		Inventaris Wob-verzoek W17-11								
			wordt verstrekt				**weigeringsgronden**			
nr.		**document NTS 20171629**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
1		Origineel aanvraagformulier				x		x		
2		NTS initieel			x					
3		Projectvoorstel initieel				x	x	x	x	
4		Bijlage beschrijving dierproeven 1 initieel				x	x	x	x	
5		Bijlage beschrijving dierproeven 2 initieel				x	x	x	x	
6		Bijlage beschrijving dierproeven 3 initieel				x	x	x	x	
7		DEC-advies				x		x		
8		Ontvangstbevestiging				x		x		
9		Verzoek om aanvullende informatie				x		x		
10		Antwoord op verzoek om aanvullende informatie				x		x		
11		NTS aangepast	x							
12		Projectvoorstel aangepast				x	x	x	x	
13		Bijlage beschrijving dierproeven 1 aangepast				x	x	x	x	
14		Bijlage beschrijving dierproeven 2 aangepast				x	x	x	x	
15		Bijlage beschrijving dierproeven 3 aangepast				x	x	x	x	
16		Verzoek om aanvullende informatie 2				x		x		
17		Bijlage beschrijving dierproeven 1 aangepast				x	x	x	x	
18		Bijlage beschrijving dierproeven 2 aangepast				x	x	x	x	
19		Bijlage beschrijving dierproeven 3 aangepast				x	x	x	x	
20		Adviesnota CCD		x						x
21		Beschikking en vergunning				x		x		

1629

1.

Centrale Commissie Dierproeven

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

03 MEI 2017

- U bent van plan om één of meerdere dierproeven uit te voeren.
- Met dit formulier vraagt u een vergunning aan voor het project dat u wilt uitvoeren. Of u geeft aan wat u in het vergunde project wilt wijzigen.
- Meer informatie over de voorwaarden vindt u op de website www.centralecommissiedierproeven.nl. of in de toelichting op de website.
- Of bel met 0900-2800028 (10 ct/min).

1 Gegevens aanvrager

1.1 Heeft u een deelnemernummer van de NVWA?
Neem voor meer informatie over het verkrijgen van een deelnemernummer contact op met de NVWA.

☒ Ja > *Vul uw deelnemernummer in* 22100

☐ Nee > *U kunt geen aanvraag doen*

1.2 Vul de gegevens in van de instellingsvergunninghouder die de projectvergunning aanvraagt.

Naam instelling of organisatie	[redacted]
Naam van de portefeuillehouder of diens gemachtigde	
KvK-nummer	
Straat en huisnummer	
Postbus	
Postcode en plaats	
IBAN	
Tenaamstelling van het rekeningnummer	

1.3 Vul de gegevens van het postadres in.
Alle correspondentie van de CCD gaat naar de portefeuillehouder of diens gemachtigde en de verantwoordelijke onderzoeker.

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	[redacted]
Functie	
Afdeling	
Telefoonnummer	
E-mailadres	

1.5 *(Optioneel)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	
Functie	
Afdeling	
Telefoonnummer	
E-mailadres	

1.6	*(Optioneel)* Vul hier de gegevens in van de persoon die er verantwoordelijk voor is dat de uitvoering van het project in overeenstemming is met de projectvergunning.	(Titel) Naam en voorletters	☐ Dhr. ☐ Mw.
		Functie	
		Afdeling	
		Telefoonnummer	
		E-mailadres	
1.7	Is er voor deze projectaanvraag een gemachtigde?	☐ Ja > *Stuur dan het ingevulde formulier Melding Machtiging mee met deze aanvraag*	
		☐ Nee	

2 Over uw aanvraag

2.1	Wat voor aanvraag doet u?	☒ Nieuwe aanvraag > Ga verder met vraag 3
		☐ Wijziging op (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn
		Vul uw vergunde projectnummer in en ga verder met vraag 2.2
		☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn
		Vul uw vergunde projectnummer in en ga verder met vraag 2.3
2.2	Is dit een *wijziging* voor een project of dierproef waar al een vergunning voor verleend is?	☐ Ja > Beantwoord dan in het projectplan en de niet-technische samenvatting alleen de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier
		☐ Nee > Ga verder met vraag 3
2.3	Is dit een *melding* voor een project of dierproef waar al een vergunning voor is verleend?	☐ Nee > Ga verder met vraag 3
		☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	1 - 9 - 2017
		Einddatum	1 - 9 - 2022
3.2	Wat is de titel van het project?	Research of new ruminant vaccines	
3.3	Wat is de titel van de niet-technische samenvatting?	Onderzoek naar nieuwe vaccins tegen ziektes bij herkauwers	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	[redacted]
		Postadres	
		E-mailadres	

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 1.541 Lege

☐ Wijziging € Lege

4.2 Op welke wijze wilt u dit bedrag aan de CCD voldoen.
Bij een eenmalige incasso geeft u toestemming aan de CCD om eenmalig het bij 4.1 genoemde bedrag af te schrijven van het bij 1.2 opgegeven rekeningnummer.

☐ Via een eenmalige incasso

☒ Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☐ Melding Machtiging

☒ DEC advies
x Factuurinformatie

6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD of per post naar:

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag

Ondertekening door de instellingsvergunninghouder of gemachtigde (zie 1.7). De ondergetekende verklaart:

- dat het projectvoorstel is afgestemd met de Instantie voor Dierenwelzijn.
- dat de personen die verantwoordelijk zijn voor de opzet van het project en de dierproef, de personen die de dieren verzorgen en/of doden en de personen die de dierproeven verrichten voldoen aan de wettelijke eisen gesteld aan deskundigheid en bekwaamheid.
- dat de dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU, behalve in het voorkomende geval de in onderdeel F van de bijlage bij het bij de aanvraag gevoegde projectvoorstel gemotiveerde uitzonderingen.
- dat door het ondertekenen van dit formulier de verplichting wordt aangegaan de leges te betalen voor de behandeling van de aanvraag.
- dat het formulier volledig en naar waarheid is ingevuld.

Naam [redacted]

Functie

Plaats

Datum

Handtekening

Centrale Commissie Dierproeven

2.

Format
Niet-technische samenvatting

- Dit format gebruikt u om uw niet-technische samenvatting te schrijven
- Meer informatie over de niet-technische samenvatting vindt u op de website www.centralecommissiedierproeven.nl.
- Of neem telefonisch contact op. (0900-2800028).

1 Algemene gegevens

1.1	Titel van het project	Onderzoek naar nieuwe vaccins tegen ziektes bij herkauwers
1.2	Looptijd van het project	5 jaar
1.3	Trefwoorden (maximaal 5)	Vaccin, immuniteit, rund, schaap, geit

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

- ☐ Fundamenteel onderzoek
- ☒ Translationeel of toegepast onderzoek
- ☐ Wettelijk vereist onderzoek of routinematige productie
- ☐ Onderzoek ter bescherming van het milieu in het belang van de gezondheid
- ☐ Onderzoek gericht op het behoud van de diersoort
- ☐ Hoger onderwijs of opleiding
- ☐ Forensisch onderzoek
- ☐ Instandhouding van kolonies van genetisch gemodificeerde dieren, niet gebruikt in andere dierproeven

3 Projectbeschrijving

3.1	Beschrijf de doelstellingen van het project (bv de wetenschappelijke vraagstelling of het wetenschappelijk en/of maatschappelijke belang)	Binnen het project wordt onderzoek verricht naar nieuwe vaccins voor herkauwers (runderen, schapen en geiten). Vaccinatie tegen infectieziekten bij herkauwers levert een belangrijke bijdrage aan het verminderen van antibiotikagebruik. Een aantal ziekteverwekkers bij de herkauwers kunnen ook bij mensen ziekten veroorzaken. Door de herkauwers tegen deze ziekteverwekkers te vaccineren, wordt ook het risiko op besmetting van de mens verlaagt. Het onderzoek is nodig om vaccins ter bescherming tegen nieuwe ziekteverwekkers waar nog geen vaccin voor is te ontwikkelen, maar ook voor verbetering van bestaande vaccins (bv aanpassing aan verandering van de ziekteverwekker in het veld, een verbeterde samenstelling of toedieningsroute of een combinatie van bestaande vaccins die het aantal vaccinatiemomenten verminderd).

Eerst wordt met onderzoek een nieuwe ziekteverwekker geïdentificeerd of kennis van een bekende ziekteverwekker uitgebreid en wordt vaccin kandidaat getest. Als deze vaccin kandidaat voldoet aan de benodigde veiligheid en werkzaamheid start de ontwikkeling. In deze fase worden studies gedaan met de nieuwe vaccin kandidaat om aan eisen voor Europese/internationale productregistratie voor nieuwe vaccins te kunnen voldoen.
Bij ontwikkeling van vaccins voor herkauwers worden ook andere diersoorten gebruikt om o.a. benodigde veiligheid te testen in andere dieren of voor ontwikkeling van laboratorium testen.

3.2 Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang?

Beter werkende vaccins zullen bijdragen aan verdere vermindering van ziektes bij het dier en verbetering van het dierenwelzijn en de groei. Tevens zal de noodzaak en gebruik van alternatieve middelen als antibiotica verminderen. Door combinatie van vaccins zal het aantal vaccinatie momenten en/of de daarmee gepaard gaande stress verminderen. In geval van vaccins tegen ziekteverwekkers die ook mensen ziek kunnen maken zal het vaccin tegelijkertijd de voedselveiligheid verbeteren als ook de veiligheid voor de veehouder en medewerkers in de melk- en vleesverwerking .

3.3 Welke diersoorten en geschatte aantallen zullen worden gebruikt?

Diersoort	Aantallen
Runderen	6565
Schapen	1220
Geiten	210
Konijnen	700
Muizen	650
Ratten	650
Cavia's	550
Kippen	700

3.4 Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren?

De dieren ondervinden licht ongerief van de entingen en bemonsteringen (bloedafname, rectal swab, neusswab etc.). Bij herhaalde bemonstering wordt het ongerief als matig ingeschaald. In het begin van een project worden infectiestudies met de ziekteverwekkers uitgevoerd om te onderzoeken welke ziekteverschijnselen en immuun reacties optreden. Op basis van deze studies worden ook de protocollen ontwikkeld om in een later stadium de werkzaamheid van een vaccin te testen. In deze infectiestudies krijgen gevaccineerde dieren ziekteverwekkers, waartegen het vaccin gericht zal zijn toegediend. In deze studies wordt ter vergelijking ook aan niet-gevaccineerde dieren dezelfde ziekteverwekker toegediend waarna deze dieren ziek kunnen worden. Afhankelijk van het ziektebeeld per ziekteverwekker zullen de ongevaccineerde dieren voor een korte periode matig tot ernstig ongerief kunnen ondervinden.
Infectiestudies kunnen ook nodig zijn om te onderzoeken of een potentieel vaccinstam afdoende verzwakt is.
Tevens worden diermodellen ontwikkeld, om de kwaliteit van de individuele batches van een vaccin voor de verkoop te controleren.

3.5 Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst?

Licht: 15 %
Matig: 76 %
Ernstig: 9 %

3.6 Wat is de bestemming van de dieren na afloop?

Doorgaans worden dieren geëuthanaseerd omdat er van een aantal weefsels ~~be~~monster~~d~~s genomen moeten worden, bv om deze onder het microscoop verder te onderzoeken. Indien dat niet het geval is kunnen, afhankelijk van de soort studie, dieren na beëindiging van een experiment teruggeplaatst

worden in de commerciële dier- en veehouderij, of hergebruikt. Ernstig zieke dieren of dieren waarbij het welzijn onverwacht is aangetast worden op een humane wijze geëuthanaseerd volgens geaccepteerde en wettelijk toegestane methoden.

4 Drie V's

4.1 **Vervanging** Geef aan waarom het gebruik van dieren nodig is voor de beschreven doelstelling en waarom proefdiervrije alternatieven niet gebruikt kunnen worden.	De werkzaamheid van een vaccin hangt af van de reactie op het vaccin door het immuunsysteem van het dier en het vermogen van het geactiveerde immuunsysteem om later in de tijd een infectie te overwinnen. Dit is een dermate complex systeem dat er geen betrouwbare vervangende test zonder dieren voor is. Waar mogelijk, dwz waar er een *in vitro* test is die correleert met bescherming en waar deze door de regulatoire autoriteiten wordt geaccepteerd, wordt hiervan gebruik gemaakt. Het is ons streven om hier zoveel mogelijk gebruik van te maken en ook in het voorafgaand onderzoek hier specifiek naar te kijken.
4.2 **Vermindering** Leg uit hoe kan worden verzekerd dat een zo gering mogelijk aantal dieren wordt gebruikt.	Voor zover van toepassing zullen de protocollen van de uit te voeren testen de richtlijnen inclusief de diersoort zoals vastgelegd in de Europese Farmacopee (wet- en regelgeving voor humane en veterinaire geneesmiddelen) of andere regulatoire regelgeving van overheden volgen. Het aantal benodigde dieren in de experimenten wordt statistisch doorgerekend, om niet te veel dieren te gebruiken maar tegelijkertijd wel de zekerheid te hebben dat de gegevens die uit het experiment komen, betrouwbaar genoeg zijn om conclusies uit te trekken ter voorkoming van herhalings-experimenten. Daarnaast worden dieren indien mogelijk opnieuw gebruikt met in acht neming van de bewaking van het dierenwelzijn en regelgeving.
4.3 **Verfijning** Verklaar de keuze voor de diersoort(en). Verklaar waarom de gekozen diermodel(len) de meest verfijnde zijn, gelet op de doelstellingen van het project.	Bij onderzoek naar nieuwe vaccins dienen de veiligheid en werkzaamheid van een product te worden aangetoond in het doeldier (in casu rund, schaap of geit). Daarnaast worden o.a. muizen, konijnen, cavia's en kippen gebruikt voor het opwekken van antilichamen en antisera voor testontwikkeling (mogelijk ter vervanging van dierstudies) en om de werkzaamheid van vaccin chargen te testen in het kader van de kwaliteitsbewaking. Indien het toepassen van veterinaire behandeling (bijv pijnstilling) niet interfereert met het experiment zal daar waar mogelijk adequate veterinaire behandeling worden toegepast. Daarnaast worden er bij alle dierproeven vooraf vastgestelde humane eindpunten gehanteerd om het ongerief en lijden van dieren zo veel mogelijk te beperken.
Vermeld welke algemene maatregelen genomen worden om de negatieve (schadelijke) gevolgen voor het welzijn van de proefdieren zo beperkt mogelijk te houden.	De instelling beschikt over adequate gebouwen en voorzieningen om in de huisvestingsbehoefte van betreffende diersoorten te voorzien en om de procedures efficiënt uit te voeren met zo min mogelijk stress bij de dieren. De dieren worden sociaal (in groepen) gehuisvest en beschikken over kooiverrijking passend bij de diersoort zodat de dieren soort-specifiek gedrag kunnen uitvoeren. Alle biotechnische handelingen en de dagelijkse handelingen voor de huisvesting en de verzorging van de dieren worden gedaan door

gediplomeerde en ervaren medewerkers.

Voor de controle en monitoring van het dierwelzijn beschikt de instelling over een Instantie voor Dierenwelzijn en gekwalificeerde dierenartsen waardoor passende veterinaire zorg te~~n~~ allen tijde beschikbaar is. Ernstig zieke dieren of dieren waarbij het welzijn onverwacht is aangetast worden op een humane wijze geëuthanaseerd volgens geaccepteerde en wettelijk toegestane methoden.

5 In te vullen door de CCD

Publicatie datum	
Beoordeling achteraf	
Andere opmerkingen	

Centrale Commissie Dierproeven

Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	22100
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	Provide the title of the project.	Research of new ruminant vaccines

2 Categories

2.1 Please tick each of the following boxes that applies to your project.

- ☐ Basic research
- X Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or
- ☐ Research aimed at preserving the species subjected to procedures
- ☐ Higher education or training
- ☐ Forensic enquiries
- ☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

Rationale

Ruminants represent around 32% of the global animal health market, with vaccines being a key segment in the ruminant health market. This market is extremely diverse: the most important species of farmed ruminants are cattle, sheep and goat, but in certain regions, also buffalo, deer, and camels are raised in

an agricultural setting. The husbandry and management systems vary between species and also on whether the animals are kept for meat, milk or wool production. By consequence, a broad and diverse portfolio of ruminant vaccines is required to fulfil the specific needs for the different markets.
One aspect is however constant across the whole ruminant health market: the trend of increasing animal productivity and growing farm/herd size, which is worsening the impact of infectious diseases. Vaccination is widely applied to control infectious diseases, leading to a reduction in the amount of antibiotics used and better wellbeing for the animals.
The company is constantly striving to strengthen its portfolio of ruminant vaccines by improving existing vaccines and developing new ones. New fields of vaccine research may include microorganisms that are by themselves harmless for ruminants, but animals are vaccinated to reduce the risk of infection for humans for example in case of [redacted] diseases or other zoonoses. Vaccination for other reasons such as [redacted] or other targets that are not infectious agents are out of scope for this application.

Each pathogen has its own specific mechanism of pathogenesis and protection through vaccination requires specific immune responses, either humoral, through cellular immunity, or a combination thereof. For this reason, new vaccines are to a large extent "tailor-made". Where possible, knowledge acquired with other pathogens / vaccines is used to design candidate vaccines in order to increase the likelihood of success and thereby minimize the numbers of animals needed during the whole development process of a new vaccine. Vaccines can be either live attenuated pathogens, (components of) inactivated pathogenes, [redacted].
Most inactivated vaccines are formulated together with an adjuvant and primarily induce a humoral response, while live (vector) vaccines typically induce both a humoral and cellular response. The attenuation of live vaccines can be accomplished by classical means (e.g. in vitro passaging or chemical mutagenesis) or by recombinant methods (GMO vaccines).

In case of diseases that affect very young animals, the mother is vaccinated in order to protect its offspring by the transfer of specific antibodies via the colostrum and milk or specific antibody preparations are added to the milk.

The two key requirements for any vaccine are (i) safety and (ii) efficacy. As vaccination is a medical treatment administered to healthy individuals, it is important that the vaccine does not cause undesired (negative) effects other than some transient minor discomfort. With regard to efficacy, the benefit in providing protection from disease and/or infection has to be demonstrated to justify the use. According to the applicable regulations in Europe and the majority of other countries, the safety and efficacy of a vaccine candidate has to be demonstrated in animal experiments. In addition, some quality control tests also require animal testing.

Vaccine research and development

Within a vaccine project two phases can be distinguished: the research phase and the development phase.
The research phase comprises the search for new pathogens, new adjuvants, new vaccine formulations and new [redacted] methods as well as expansion of the knowledge on known pathogens and technologies. For example, mechanisms of pathogenicity and natural and specifically induced immune responses or strategies of the pathogens to [redacted] will be investigated, protective antigens might be identified or methods of attenuation of strains to create live vaccines might be investigated. Candidate vaccines are then tested in pilot studies to determine their efficacy and safety. Based on the outcome of these studies, candidate vaccines with a fixed formulation are selected to enter the development phase.
In addition, (antigenic components of) the pathogens will be used to immunize laboratory animals to generate antibodies that can be used to set up immunological assays that are needed during the development phase
The current project proposal covers studies that are done during the research phase. The development phase is covered by a separate project proposal.
In general, the design of the development studies, including the procedures that have to be applied to demonstrate safety and efficacy of the vaccine are laid down in Guidelines and Regulations. The design and the procedures applied in the research studies are basically the same as for the development studies:

- Infection studies with new pathogens are performed to set up target animal infection models that can be used in efficacy studies in the development phase and to expand the knowledge on these

pathogens with regard to pathogenesis, immune responses, etc.
- Safety and efficacy research studies are performed in order to select vaccine candidates that are likely to successfully pass the development studies
- The objective of studies on *in vivo* potency testing during the research phase is to set up a test protocol for an in vivo potency test that can be applied during the development phase.

During the research phase, less knowledge and experience is available on the different pathogens / vaccine candidates / adjuvants. Therefore, higher discomfort scores may be reached in a larger proportion of animals.

A distinct type of studies is the immunization of laboratory animals to generate specific antibodies that can be used for immunological assays that are needed the indentification, detection and/or quantification of the antigen/pathogen. The commonly applied design and procedures are followed in these studies.

Below, some back-ground information is given about infectious diseases in ruminants that are in scope for this project.

Diseases within the ruminant vaccine development project

Currently, the main target species for ruminant vaccines are cattle, yet several cattle pathogens also affect sheep and goat. In these cases, testing in small ruminants might also be necessary, especially in case of zoonoses or animal pathogens that are subject of control programs. Moreover, small ruminants might serve as model for infection studies.

The following infectious diseases are targeted within the ruminant vaccine development projects in the company.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

A) [redacted]

[redacted]

[redacted]

3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

The goal of the ruminant vaccine research project is to update the current [redacted] vaccine portfolio in response to unmet needs in the field of ruminant livestock industry. More specifically, the aim is to identify new pathogens, expand the knowledge of known pathogens to be able to develop new (combination) vaccines and test candidate vaccines against these pathogens. The outcome of a successful research phase is the discovery of a new pathogen and/or a prototype vaccine that has shown to be safe and efficacious in the target animals (proof of concept) and will therefore later on successfully pass the development phase. In addition, (antigenic components of) the pathogens will be used to immunize laboratory animals to generate antibodies that can be used to set up immunological assays for the detection and/or quantification of the antigen/pathogen and the immune response against the antigen/pathogen (e.g. ELISAs, serotyping tests, immunohistochemistry, humoral and cellular immune responses).

3.3 Relevance

What is the scientific and/or social relevance of the objectives described above?

Vaccines are the most effective method for prevention or eradication of diseases. Further improvement and extension of the available vaccine range will bring safer, more efficacious vaccines, including

vaccines against emerging diseases. Also, combinations of diseases can be encountered more effectively, with fewer vaccination moments (injections), when vaccines are developed that can be used at the same time or mixed with other vaccines, or even in ready to use combination products.
The prospects are that the new vaccines will further reduce animal suffering and the use of antibiotics, and will lead to reduced losses in meat and milk production and thereby to a more sustainable use of natural resources.
Acquired insight in pathogensis and immune responses of infections will help to identify new vaccination strategies for those pathogens for which no efficacious vaccine exists at this moment.

3.4 Research strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

Infection studies in the target animal (ruminants) have to be undertaken to show that a (newly isolated) infectious agent is pathogenic and fulfils Koch's postulates and to better understand the mechanisms of pathogenicity and immune response after infection. These infection studies can also form the basis for a target animal (ruminants) infection model that will be used to test the efficacy of vaccine candidates (vaccination-challenge studies). Such an infection model is needed to be able to show that a vaccine is capable to prevent or significantly reduce infection and/or clinical signs. For vaccines against some pathogens, the infection model that has to be used and the specific efficacy criteria that have to be fulfilled are prescribed in a monograph of the Ph.Eur. When doing (initial) vaccination-challenge studies, it is attempted to find an [redacted] so that in further studies efficacy can be evaluated on the basis of e.g. the [redacted]
Safety of vaccine candidates has also to be evaluated in the target animals (ruminants) to show that systemic and local (injection site) reactions after vaccination, if any, are acceptable. For each new vaccine, a risk-benefit analysis has to be made and the aim is to induce as little as possible discomfort by vaccination.
In the research phase, safety and ([redacted] efficacy parameters are measured simultaneously in combined orientating efficacy and safety studies.

3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

The research phase for new vaccine consist of one or more of the following types of animal experiments (described in detail in appendices 1 through 3).

Ad 1) Infection studies
An infection model for a pathogen is developed based on the scientific literature or a Ph.Eur monograph (if available) and the experience with other pathogens within the company. In a model, it will be attempted to reproduce the clinical signs that are associated with a certain disease or syndrome. For a potentially new pathogen this will reveal if it is indeed able to induce disease (Koch's postulates). For known pathogens the model has to allow assessment of the efficacy of vaccine candidates under controlled laboratory conditions as described in Ph.Eur 5.2.7 (Evaluation of efficacy of veterinary vaccines and immunosera) or vaccine specific monographs, EU Directive 2009/9/EC amending Directive 2001/82/EC (Community code relating to veterinary medicinal products) and national guidelines and regulations outside the EU. New infection models are defined as models for newly discovered (potential) pathogens or published models that have not been used within [redacted] before. Improvement or refinement of existing models will be undertaken in case not all disease characteristics that are relevant for the field situation are presented in the model, or if the model shows a high variability in the level of infection/pathology within a group of infected animals and which would therefore necessitate the inclusion of large groups of animals in infection-challenge experiments in order to be able to show statistically significant differences. In case of [redacted] diseases, it might be necessary to mimic the [redacted] effect of [redacted] factors in order to reach the required sensitivity of the animals to the challenge infection with a single pathogen.For this purpose, [redacted] drugs might be applied or the animals are co-infected with [redacted] pathogens.
Testing of new serotypes/pathotypes of a pathogen is also considered improvement rather than development of a new model, as the route of application etc. will be based on experience present within the company. Furthermore, refinement will also include the testing of modifications to a model with the intention to increase animal welfare (e.g. a less invasive application method).

Studies to investigate the pathogenicity and / or to develop or improve an infection model will have the following set-up:

- Administration of a (potential) pathogen, if required in combination with [redacted] treatment
- Observation of clinical signs post infection/sampling (e.g. for shedding of the pathogen, immune responses against the pathogen)
- Necropsy to investigate (histo)pathological changes

The degree of discomfort will depend on the nature of the pathogen involved as the infection model is supposed to mimic the natural disease as much as possible.

Ad 2) Vaccination studies

Once an infection model has been established, the efficacy of candidate vaccines against a pathogen can be evaluated. When looking into the options for vaccination against a newly discovered pathogen or a pathogen for which no vaccine is available, the vaccine candidate(s) to be tested are based on the scientific literature and the knowledge within the company on pathological processes, immune mechanisms and vaccines against related pathogens to have the highest chance of success and thereby minimize the number of animals needed. This will determine whether a live or inactivated vaccine approach will be taken. In some instances, there will be collaboration with outside partners (e.g. universities) that have specific knowledge on a (new) pathogen and that might even already have prepared and tested vaccine candidates. In addition, research on new (combination) vaccines for pathogens that are already being controlled by vaccination will also be guided by the experience gained under field conditions with the marketed product(s). An inactivated vaccine can be a whole killed microorganism or virus, or an immunogenic part of the pathogen (subunit vaccine). Also [redacted] vaccines or vaccines [redacted] fall into this category. An inactivated vaccine will be formulated with an adjuvant that is expected to be safe in the target animals (ruminants) and if applicable the laboratory animal used for the potency testing in combination with the chosen antigen(s) and will be quality control tested (e.g. for sterility) before the start of an animal experiment to reduce the chances of unwanted vaccination reactions. A live vaccine is an attenuated form of the pathogen that has been prepared by "classical methods", such as cell culture passage or chemical mutagenesis, or by targeted gene modifications with the help of recombinant-DNA techniques. Another form of live vaccines, are so called vector vaccines that consist of either or non-pathogenic microorganisma or an attenuated pathogen that also contains antigen(s) of other pathogens. A live vaccine candidate will be characterized and tested for purity before the start of studies in animals. In vaccination-challenge studies using the infection model, it will be evaluated whether the vaccine candidate can provide the required protection against the pathogen in terms of reduction of infection, clinical signs and (histo)pathology. Only if a vaccine candidate gives promising results (i.e. (statistically significantly) reduces one or more aspects of a disease) it is considered for the development phase. By studying the immune response after vaccination, it will be attempted to find a correlation between the height of the immune response (e.g. as measured in in vitro virus-neutralization) and protection in the target animal (ruminant). In those instances where such a correlation can be established, candidate vaccines can be tested on the basis of that response instead of by challenge infection. However, although some vaccines are able to protect against the disease in question, the immune response measured (if any) is not always indicative of the level of protection, especially in case protective antigen(s) are unknown. If no correlate of protection is available, vaccine efficacy can only be evaluated in vaccination-challenge studies.

In order to make a proper risk-benefit analysis for a new product, all vaccines have to be tested in safety studies in the target animal (Ph.Eur 5.2.6 (Evaluation of safety of veterinary vaccines and immunosera), EU Directive 2009/9/EC amending Directive 2001/82/EC (Community code relating to veterinary medicinal products), VICH guidelines and national guidelines and regulations). Inactivated and subunit vaccines usually contain an adjuvant that enhances the immune response to the antigen(s) in the vaccine. Unfortunately, although the adjuvant preparations themselves can be considered safe, the combination of antigen and adjuvant sometimes results in unwanted systemic and/or local reactions after vaccination. Therefore, for each new inactivated or subunit vaccine the effect on the animals' general health, determined by observing clinical signs (e.g. general demeanour, body temperature, appetite etc.) and injection site reactions has to be determined. For an attenuated live vaccine, it has to be shown that it is unable to induce disease. Therefore, live vaccine candidates will be evaluated for their lack of virulence in the infection model. Testing specific gene-deleted mutants in the infection model will also provide knowledge on which antigens are required for pathology and/or survival within the host. These antigens can then be considered for an inactivated vaccine approach.

As evaluation of the safety and efficacy of vaccination will be combined in the research phase, studies will be performed according to the following basic set-up (infection will not be performed in case an

immunological marker for protection can be applied):
- Administration of the candidate vaccine
- Observation of clinical signs post vaccination
- Monitoring responses (e.g. blood sampling)/persistence (e.g. shedding) in case of a live vaccine
- Infection with a pathogen (field isolate)
- Observation of clinical signs post infection/sampling (e.g. for shedding of the pathogen)
- Necropsy to investigate (histo)pathological changes

The degree of discomfort encountered directly as a result of vaccination and sampling is small and such procedures are routinely used in normal veterinary care. The one area in which a moderate to severe degree of suffering may occur is after the onset of clinical disease following infection.

Ad 3) Assay development and preparation of biomaterials
To set-up assays that can help to detect a pathogen (e.g. by immunohistochemistry),to discriminate between different strains (e.g. serotyping) or develop immunological assays to quantify whole pathogens or specific antigens (antigenic mass and potency tests) it may be necessary to use laboratory animals for the preparation of sera or monoclonal antibodies if the required reagents are not available. For each new vaccine, batch tests for the quantification and identification of the active ingredients are required under EU Directive 2009/9/EC amending Directive 2001/82/EC (Community code relating to veterinary medicinal products) and Ph.Eur 0062 (Vaccines for veterinary use) to verify the consistency of the manufacturing process and the final product. Preferably, in vitro tests are used for batch testing, but in case an in vitro batch potency test is not possible for a new vaccine, a serological assay in laboratory animals will have to be set up. In addition, for some vaccines for which a Ph.Eur monograph exists, a mandatory batch potency test in laboratory animals is described.
In a few of cases, determination of the protection against challenge infection is mandatory or necessary for scientific or technical reasons.

Immunization experiments of laboratory animals will generally be as follows:
- Administration of antigen/pathogen
- Collection of blood

In case of potency tests that involve challenge infection, the following additional treatments are employed
- Administration of challenge inoculum
- Clinical observation

The degree of discomfort encountered directly as a result of vaccination and sampling is small and such procedures are routinely used in normal veterinary care. In the studies without challenge, discomfort will be mild or moderate depending on the number of injection/sampling moments.
Moderate to severe degree of suffering may only occur in studies with challenge infection.

3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points.

At the company, all R&D projects are subject to regular review by the R&D Governance Body. Only research projects with a reasonable market expectation will get approval to start the research phase. At the start of a research project, the project team agrees on the types of studies to be performed, the requirements for the studies and the sequence of these studies.

To test the feasibility of vaccine development against a (new) pathogen, the sequence of experiments is the following:

1. Infection studies.

The appearance of new strains of a particular pathogen is often difficult to observe, and can rely on anecdotal reports from veterinarians and animal owners. Field isolates might therefore have to be tested both in vitro and in vivo to determine if significant changes in pathogenicity have occurred. Studies are performed to investigate the pathogenicity of an agent and the immune response induced by the agent. If a potential pathogen fails to fulfil Koch's postulates, the vaccine research project will be stopped. The infection studies also serve to develop or improve an infection model. Once an infection model is available it can be used for fundamental studies and the next steps for vaccine development. These infection models should mimic the natural disease as much as possible. In case of [redacted] diseases, it might be necessary to [redacted] effect of [redacted] factors in order to reach the required [redacted] of the animals to the [redacted] with a [redacted]. As it is important to have the smallest possible variation in the level of infection/clinical signs between animals to be able to work with the lowest number of animals possible in the challenge experiments performed during the development phase, improvement/refinement of the challenge model (e.g. change

in the route of inoculation) may need to be undertaken. Also, when a new challenge inoculum is prepared, suitability for use in challenge studies has to be evaluated in the model.
On the other hand infection studies are required to determine whether a strain is suffiently attenuated to serve as vaccine strain. On the basis of the study results, it will be determined if a live vaccine candidate has an acceptable risk-benefit profile. Some fine-tuning of the composition (e.g. changes in dose or application route) may be necessary before the optimal vaccine has been reached (proof of concept). In some cases it may not be possible to obtain proof of concept with the available candidates and knowledge of the pathogen, which means that the research project will be stopped.

2. Vaccination challenge studies

Orienting vaccination challenge studies are performed to get a first impression about the safety and efficacy of the vaccine candidates. It may take several rounds of experiments to test a number of vaccine candidates in order to find a candidate vaccine that has proven to be able to fulfil the required efficacy and safety criteria, the vaccine candidate can move to the development phase. If, with the knowledge available, it is impossible to produce a candidate vaccine that fulfils the criteria, further development is stopped.

3. Studies in laboratory animals for assay development and preparation of biomaterials.

These will only be undertaken if the necessary immunological reagents are not (commercially) available. A potency test in laboratory animals (according to Ph.Eur monographs) will only be developed in case a no suitable *in vitro* test is available.
All above mentioned studies are eventually done to obtain regulatory approval for ruminant vaccines. During the regular (1-2 times per year) project reviews by the R&D Governance Body the outcome of the studies is assessed against the requirements and pre-set milestones as well as go/no-go decision points are evaluated.

3.4.4 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

Serial number	Type of animal procedure
1	Infection studies in ruminants
2	Vaccination challenge studies in ruminants
3	Assay development and preparation of biomaterials
4	
5	
6	
7	
8	
9	
10	

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	22100
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 1 — Type of animal procedure: Research: Infection studies in ruminants

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

Infection studies will be performed for one of the three different reasons:

1) To determine the pathogenicity of new pathogens (to prove Koch's postulates), variants of known pathogens, should they appear or the role of specific genes in the pathogenicity and interaction with the host's immune system.
2) To develop an infection model that will be used in vaccination-infection studies to assess the efficacy of vaccine candidates (see Addendum 2).
3) To assess the safety profile of a live attenuated vaccine candidate obtained by "classical" means (e.g. in vitro passage or chemical mutagenesis) or gene-modification (including vector vaccins).

In general, the application of a pathogen will be done via the natural route of infection, but if the natural route does not induce all presentations of a disease under laboratory conditions, it might be necessary to use another route (e.g. parenteral injection to induce a systemic infection).
Application of a potential vaccine candidate will typically be done by the route intended as application route of the future product, but in specific cases it might be necessary to follow the route that gives highest risk of adverse events in order to make a meaningful assessment of the safety profile.
In case a pathogen or vaccine candidate might cause transplacental infection, pregnant animals at one or more specified stage of pregnancy have to be used in the infection studies. The animals are then observed until the end of pregnancy to determine the outcome of the pregnancy and the health status of the offspring. Samples might have to be taken from the offspring to determine the presence of the pathogen / vaccine candidate or specific pre-colostral antibodies. Alternatively, [redacted].

After inoculation of the vaccine candidate or pathogen, one or more of the following parameters will be

evaluated:

- Clinical signs (e.g. changes in general health and or disease specific symptoms and local reactions)
- Body temperature (rectal temperature)
- Body weight
- Virus, bacterial or parasites shedding (swabbing of mucosal surfaces, sampling of faeces, urine, milk), viral/bacterial/parasitic load in [redacted] or other tissues ([redacted])
- Viraemia, bacteraemia or parasitemia or haematological parameters (blood/ [redacted] sampling)
- Post mortem examination (macroscopical and microscopical)

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Four or more of the following procedures will be undertaken depending on the characteristics of the vaccine/ pathogen involved; the types of pathogens are described under B (in italics the frequency of the procedures):

1. Daily observation / scoring clinical signs including measurement of rectal temperature [redacted] *)*
2. Weighing *(*[redacted]*)*
3. Blood sampling *(*[redacted]*)* to determine [redacted] parameters and / or to determine the presence of the pathogen or vaccine candidate in the blood
4. [redacted] treatment by application of [redacted] drugs ([redacted] / [redacted]) and / or inoculation of [redacted]
5. Administration of pathogen or vaccine candidate ([redacted]
6. Swabbing of (mucosal) surfaces [redacted] to determine the excretion of the pathogen or vaccine candidate
7. [redacted] to determine the presence of the pathogen or vaccine candidate in the [redacted]
8. [redacted]) *(1x – 4x)* to determine the presence of the pathogen or vaccine candidate in the [redacted]
9. Urine, fecal, colostrum / milk samples [redacted] to determine the presence of the pathogen or vaccine candidate
10. Punction of [redacted] or installation of a [redacted]
11. Punction [redacted]
12. [redacted] biopsy [redacted]
13. Pregnancy check [redacted]
14. Euthanasia

The duration of all procedures listed above will only be minutes at most. Typically, the length of the observation period after infection is [redacted]. If pregnant animals have to be followed until birth, the observation period will be longer, but in general, the total number of treatments does not increase.
To rule out that clinical signs are caused by an unintended co-infection, non-infected control animals may be included in a study. In addition, in models for neonatal disease in sheep and goats, ewes/goats may be required to give birth to and foster the lambs / kids but these will not be infected.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

In this type of initial studies, it is not obligatory to demonstrate statistically significant differences between treatment groups. However, these studies will enable an estimation of the variance between individual responses of the animals in a group at these particular observation points. Based on this information the minimum numbers of animals per group needed to demonstrate the efficacy of a vaccine during the development phase can be estimated. In this type of experiments, the group size is in general 5-8 animals, depending on the expected variation in the infection model. This group size is in line with the group size for infection studies as specified in most European Pharmacopoeia monographs on ruminant vaccines.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Studies will be performed in cattle, sheep and / or goats as appropriate. Animals of both sexes can be used for this type of animal experiment unless the study has to be performed in pregnant and / or lactating animals.

Purchase of animals: The animals will be purchased from commercial suppliers, obtained from affiliated farms or bred at [redacted] facilities.
If a certain microbial status is required, animals will be purchased from farms with the respective (certified) microbiological status and / or screened prior to inclusion in the study.
If the study design requires that animals are vaccinated and / or infected at [redacted] or when only a [redacted], the young animals have to be [redacted] accordingly from the farms of birth to the testing facility.

Special requirements
If young animals with a specific [redacted] status are required, it is often necessary to warrant that [redacted]. As these [redacted] animals are more susceptible for intercurrent infections, they receive [redacted] treatment. With the exception of studies against [redacted] pathogens, [redacted] can be given to the animals [redacted] onwards as an aid in the prevention of [redacted]

Age of animals:
Age of the animals for vaccine research varies from a few hours old to adult. The age of the animals to be used should be the age at which clinical disease is expected or the minimal age recommended for use of the vaccine.
If the infection has to be done in very young lambs or kids, it might be necessary to include the ewes / goats to foster the lambs / kids. In order to study transplacental spread of the pathogen or vaccine

candidate it may be necessary to include dams in one or more specific trimester of pregnancy. Samples might have to been taken from the offspring, for certain studies, euthanasia of the offspring might be required.

The tables below specify which models would be used for the different pathogens that might be included in infection studies during the research projects over the next 5 years. The lists are more extensive than the actual portfolio will be, but it is not possible at this moment to predict exactly which pathogens will be worked on.
The animal categories listed are the age groups considered to be most sensitive and therefore have to be used to perform the basic efficacy and safety studies.

Priorities within the R&D portfolio are based on market needs and the estimated likelihood of success of obtaining a vaccine candidate that fulfills the required product profile. Priorities can shift upon identification of new unmet needs in the field. For example, if a new [redacted] pathogen with substantial impact on the ruminant industry is discovered, this will be given priority over research into a second generation improved product for a [redacted] pathogen.

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 2 wk
New pathogen	Cattle <6 Months old	Severe (max 70%)[1]	Max 2 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 2 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 2 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 50%)	Max 1 2 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
New pathogen	Cattle <6 Months old	Moderate (max 100%)[2]	Max 2 wk

[1]Vaccines are given to [redacted] cattle in order to [redacted]. This part of the study does only cause mild discomfort. The information given in the table above relates to the discomfort during challenge studies [redacted] will be determined.

[2]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 50%)	Max 2 wk
[redacted]	Adult cows	Moderate (max 50%)	Max 2 wk
[redacted]	Adult cows	Mild	Max 2 wk
[redacted]	Adult cows	Mild	Max 2 wk
[redacted]	Adult ewes	Mild	Max 2 wk
[redacted]	Adult ewes	Mild	Max 2 wk
New pathogen	Adult cows	Moderate (max 100%)[1]	Max 2 wk
New pathogen	Adult ewes	Moderate (max 100%)[1]	Max 2 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk

[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
New pathogen	Adult cows	Severe (max 100%)[1]	Max 1 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle <6 Months and adult cows	Moderate (max 50%)	Max 2 wk

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	sheep <6 Months	Severe (max 40%)	Max 1-2 d
	cattle<6 Months	Moderate (max 70%)	Max 2 wk
[redacted]	sheep <6 Months	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 70%)	Max 1-2 days
[redacted]	sheep <6 Months	Severe (max 50%)	Max 1 wk
[redacted]	cattle<6 Months	Moderate (max 70%)	Max 1 wk
[redacted]	sheep <6 Months	Mild	Max 2 wk
	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Severe (max 50%)	Max 1-2 d
[redacted]	sheep < 6 Months	Severe (max 50%)	Max 1-2 d
[redacted]	cattle<6 Months sheep < 6 Months	Mild	Max 2 wk
New pathogens and new types of models [redacted]. [redacted])	cattle<6 Months sheep < 6 Months	Severe (max 100%)[2]	Max 1 w

[1] Efficacy of [redacted] vaccines in ruminants is tested by [redacted] only

[2]: Studies with potential new pathogens are given the maximum discomfort score until the severity of clinical signs has been established

Based on the experience over the last 5 years and the current R&D program and priorities, the total expected number of cattle, sheep and goat per age group and discomfort category is the following:

Species	Discomfort score*	Animals<6 months	Adult**
Cattle	Mild***	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]
Sheep	Mild***	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]
Goat	Mild***	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]

*: Discomfort due to disease
**: [redacted]
***: Due to repeated procedures the overall discomfort will be moderate

In the infection studies, one or more groups are compared to an uninfected control group. The group size is dependent on the disease model. In general, the group size used will be 5-8 animals but could be larger depending on the expected variation in the infection model. The expected numbers per category of vaccine are:

Per Model	Cattle <6Months	Adult cattle	Sheep <6Months	Adult sheep	Goat <6Months	Adult goat
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

C. Re-use

Will the animals be re-used?

☐ No, continue with question D.

X Yes > Explain why re-use is considered acceptable for this animal procedure.

Re-use might be considered for animals that experience only minor discomfort during the first study, for example uninfected control animals. This approach is considered acceptable especially in case of animals that were raised under special conditions (e.g. [redacted]) to achieve a certain [redacted] status

Are the previous or proposed animal procedures classified as ‘severe’?

xNo

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:
In accordance with international regulations, animals of the target species must be used to demonstrate the safety and efficacy of a vaccine because there are no suitable alternatives or models for the induction of immunity in a whole organism or for the infection of living tissues as complex as those found in the whole animal in which the vaccines are intended to have efficacy. Therefore, during the research phase, infection models have to be developed in the target animals-and candidate vaccines have to be tested in the same models.

Reduction: All studies are performed with the lowest possible number of animals that are required to enable meaningful interpretation of the results. This will be achieved through an ongoing evaluation of the observations in each study. The number of animals per study will be substantiated in each study protocol. According to internal procedures, the study protocol will be reviewed by the Animal Welfare Body and a statistician.

Refinement:
Where possible it is pursued to refine the routes of administration of substances and sampling techniques to improve animal welfare/to reduce discomfort of administration, but without endangering the scientific outcome. Ruminants are the target species and there are no other less innervated/sentient species that could be a model for the ruminant diseases that are studied. See next paragraph for other refinement methods that are applied.
The classic method to prove protection of a new vaccine is efficacy in a vaccination-challenge test. However, if immunological correlates of protection (e.g. a serological response) can be used to prove efficacy this will be used rather than challenge infection. When an infection model has to be used, humane endpoints will be employed and staff will be fully trained to recognize animals that experience discomfort. Animals will be closely monitored and additional health checks are performed to ensure that no animal is left suffering.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

For monitoring of the clinical health status of animals, all study animals will be checked at least once a day by a certified person. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.
Furthermore efforts are made to optimally enrich the environment during containment.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

The vaccines in the companys R&D program are unique and proprietary to the company. To show that vaccines are compatible (combined or associated use), a number of safety and efficacy studies done with the individual products has to be repeated with the vaccines administered together according to international regulations and guidelines.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[] No

X Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

The animals are housed socially, but animals might have to be housed (temporarily) individually without physical contact (but in the same holding room) in order to [redacted]. Some studies may require limited bedding during containment. In a few cases, it is necessary to use no bedding because of scientific reasons.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

XYes > Will anaesthesia, analgesia or other pain relieving methods be used?

[] No > Justify why pain relieving methods will not be used.

Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Injections (for application of challenge material, injection with [redacted] drugs or agents) as well as sampling of blood are part of normal farm practice/veterinary care and will induce only mild discomfort. If the sampling is repeated (>5), the discomfort is considered to be moderate as a result of the stress when restrained and during handling of the animal.
All biotechnical procedures such as vaccination and blood sampling procedures have been described in Standard Operating Procedures (SOPs) (GLP accredited procedures).

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Measurement of body temperatures and body weight, sampling urine, feaces, colostrum/milk, sampling on different mucosae [redacted], as well as [redacted] are part of normal farm practice/veterinary care and will induce only mild discomfort. If the sampling is repeated ([redacted] for the [redacted] and [redacted] or [redacted] for the other procedures), the discomfort is considered to be moderate as a result of the stress during the fixation and handling of the animal.
All biotechnical procedures such as inoculation and sampling procedures have been described in Standard Operating Procedures (SOPs) and only well trained personnel will be responsible for the execution (GLP accredited procedures).
[redacted] deprived animals are more susceptible to disease and might therefore encounter discomfort related to intercurrent diseases. Transport of the animals to the testing facility might cause transient discomfort for the duration determination the protection against challenge infection of the transport, especially for animals that are transported [redacted]
The pathogenticiy of the pathogen / safety profile of the vaccine candidate used in the infection studies is not yet determined. Therefore, it is possible that adverse events occur.
Depending on the nature of the pathogen / vaccine candidate the discomfort of the infection can range from mild in the absence of any clinical signs (e.g. [redacted]) to moderate (e.g

[redacted]) and severe (e.g. [redacted]).

In case an infection model has to be developed for pathogens that do not cause clinical disease or only very mild disease, it might be necessary to [redacted] the animals. These procedures and any effects related to the [redacted] treatment are already taken into consideration for the discomfort levels and durations listed above in the respective tables.
The clinical health status of all animals is checked at least once a day by qualified personnel. Special attention is paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.
In consultation with the veterinarian and Study Director, if treatment does not interfere with the test results, it will be decided whether to apply adequate veterinary care including analgesia to alleviate treatment related pain (for example infection studies with [redacted] pathogens or [redacted] or pain not related to the treatment. In case of severe suffering, humane endpoints are applicable. General humane endpoints are described in an SOP (e.g. the condition of the animal prevents it from eating and drinking regularly, severe loss of body weight) and test specific humane endpoints are given in each study protocol if applicable.

Explain why these effects may emerge.

These procedures may be part of the experimental design. For vaccines intended for use in young animals, the study design may require that animals are vaccinated and / or infected at the day of birth or when only [redacted]. In these cases, the young animals have to be [redacted] accordingly from the farm of birth to the testing facility.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

All biotechnical procedures will only be performed according to standard procedures described in SOPs (GLP accredited procedures).
The number of samplings is reduced to a minimum number required to for a valid evaluation of results.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

The severity of discomfort is depending on the nature of the pathogen (see 3.1 of the project proposal for specific clinical signs of the pathogens involved). However, the duration of severe discomfort will be limited due to the application of a humane endpoint if needed. Therefore, pathogen specific humane endpoints are formulated when discomfort is foreseen. These contain information about the clinical signs that can be expected and describe when a humane endpoint has been reached for (a combination of) the respective clinical signs. It also describes the scientific endpoint; the degree of progress of disease that is necessary to be able to draw conclusions about the effectiveness of the treatment tested. The humane endpoints are always leading. These test-specific humane endpoints are described in the corresponding study protocol. Each study protocol is reviewed by the AWB before execution of the study. In case it is difficult to reach a decision based on the pre-defined criteria for an endpoint the designated veterinarian is empowered to decide that a humane endpoint is applied/reached.
General humane endpoints (e.g. the condition of the animal prevents it from eating and drinking regularly, severe loss of body weight, pain) are described in an SOP. These endpoints are applicable to all animals, irrespectively of the type of experiment.

Indicate the likely incidence.

Considering the expected number of studies with the different pathogens, the expected number of animals included in the different treatment groups (i.e. infected vs control group) and the expected severity, at most 30 % of the animals is expected to have severe discomfort that would require euthanasia.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

For the infection studies the type and severity of the clinical signs are depending on the type of disease.

Similar to natural field infections they may cause mild to severe pain, distress, suffering or even impending death. See B for an overview of the different pathogens involved, the maximal discomfort caused by the disease and the maximum number of animals expected to reach the highest discomfort category.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Postmortem investigation can be part of the experimental design to evaluate (histo)pathological lesions at different organ systems and or to attempt re-isolation of the inoculum from tissues and organs.
In addition, animals with a pathogen cannot be returned to the farm of origin or transported to another farm to prevent the spread of disease. Therefore, all animals might have to be euthanized at the end of the study or when a humane endpoint is reached. Control animals that have not been infected may be reused or returned to the farm of origin or transported to another farm.
Moreover, return of the animals to commercial farms or slaughter for human consumption is often prohibited by the current legislation on use of antibiotics.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	22100
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: Research: Vaccination challenge studies in ruminants

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

During the research stage of a vaccine project, studies are carried out to estimate, whether the candidate vaccine is likely to successfully pass the safety and efficacy studies that are later on performed in the development phase.
The aim of this type of vaccination studies is to test if a vaccine candidate i) has acceptable safety characteristics and ii) is efficacious i.e. induces a measurable immune response and / or protects against one or more aspects of the disease caused by the pathogen(s) involved. To this end, animals are vaccinated according to the anticipated schedule and observed for local and systemic reaction after vaccination. The immune response after vaccination is determined. In the absence of a correlate of protection, the vaccinated animals or in case the vaccine is intended for the induction of passive protection, animals fed colostrum from vaccinated mothers will be infected with a challenge strain according to the previously established infection model (see [redacted] Unvaccinated ~~a~~animals will also be included in the experiment as control to determine the efficacy of the experimental vaccine. After vaccination and after challenge infection, one or more of the following parameters will be evaluated:

- Clinical signs (e.g. changes in general health and or disease specific symptoms and local reactions)
- Body temperature (rectal temperature)
- Body weight
- Virus, bacterial or parasites shedding (swabbing of mucosal surfaces, sampling of faeces, urine, milk), viral/bacterial/parasitic load in [redacted] or other tissues [redacted])
- Viraemia, bacteraemia or parasitemia or haematological parameters (blood/[redacted] sampling)
- Post mortem examination (macroscopical and microscopical)

On the basis of the study results, it will be determined if a vaccine candidate has an acceptable risk-benefit

profile that is in line with criteria that have been laid down in EU directives, the Pharmacopoeia Europaea (Ph.Eur) and guidelines and regulations of the European Medicines Agency and other international regulatory bodies when applicable. Some fine-tuning of the composition (e.g. changes in antigens/adjuvant included) may be necessary before the optimal vaccine has been reached (proof of concept). In some cases it may not be possible to obtain proof of concept with the available candidates and knowledge of the pathogen, which means that the research project will be stopped.
During these orientating vaccination studies, it will also be attempted to find a serological correlate with protection or another surrogate immunological marker that will enable the drawing of conclusions on the efficacy of a candidate vaccine without challenge in further studies.
In case of [redacted] diseases, it might be necessary to mimic the [redacted] effect of [redacted] factors in order to reach the required sensitivity of the animals to the challenge infection with a [redacted] pathogen.
In case a vaccine is intended for protection against transplacental infection, pregnant animals are challenged at one or more specified stage of pregnancy. The animals are then observed until the end of pregnancy to determine the outcome of the pregnancy and the health status of the offspring. Samples might have to be taken from the offspring to determine the presence of the challenge strain or specific pre-colostral antibodies. Alternatively, the vaccinated dams might have to be euthanized at pre-set times after the challenge infection in order to harvest the fetus and test fetal tissues and organs for the presence of the vaccine strain or specific antibodies.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Four or more of the following procedures will be undertaken depending on the characteristics of the vaccine/ pathogen involved; the types of pathogens are described under B (in italics the frequency of the procedures):

1. Daily observation / scoring clinical signs including measurement of rectal temperature [redacted]
2. Weighing [redacted]
3. Blood sampling [redacted] to determine [redacted] parameters and / or to determine the presence of the vaccine strain in the blood and / or to determine the presence of the challenge strain in the blood
4. Vaccine administration [redacted] / [redacted]
5. Palpation of the injection site [redacted]
6. [redacted] by application of [redacted] drugs [redacted]
7. Challenge administration [redacted]
8. Swabbing of (mucosal) surfaces [redacted] *after vaccination)* to determine the excretion of the vaccine and / or [redacted]) to determine the excretion of the challenge strain
9. [redacted] to determine the presence of the challenge strain in the [redacted]
10. [redacted] to determine the presence of the challenge strain in the [redacted]
11. Urine, fecal, colostrum / milk samples [redacted] to determine the presence of the challenge strain
12. Punction of [redacted] or installation of a [redacted]
13. Punction [redacted]
14. [redacted] biopsy [redacted]
15. Pregnancy check [redacted]
16. Euthanasia

The duration of all procedures listed above will only be minutes at most. Typically, the length of the observation period after vaccination is 14 days after each vaccination. If pregnant animals have to be followed until birth, the observation period will be longer, but in general, the total number of treatments does not increase.
The interval between vaccination and challenge infection or end of the study (in case of surrogate marker for protection) will be chosen in such a way that optimal protection is to be expected. The length of the observation period after challenge infection depends on the incubation period of the pathogen, but is

generally 1 to 4 weeks. To rule out that clinical signs are caused by an unintended co-infection, non-infected control animals may be included in a study. In addition, in models for neonatal disease in sheep and goats, ewes/goats may be required to give birth to and foster the lambs / kids but these will not be infected.

[redacted]

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

For new untested vaccine candidates it needs to be proven that they fulfil the required efficacy and safety criteria. Therefore, in initial studies small numbers of animals will be used that may not be sufficient to demonstrate statistically significant differences between treatment groups. However, with such studies it will be possible to gain an estimate of the variance between individual responses of the animals in a group at these particular observation points. This information will enable calculations to identify the minimum numbers of animals needed in the groups to give sufficient likelihood of obtaining a statistically significant result by which it can be judged that the treatments have had a real effect. In particular, the variance in the groups together with the magnitude of effect will be used in power calculations to achieve 80% power at the 95% confidence level (regarded by regulatory authorities as the standard by which such experiments should be designed).

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Studies will be performed in cattle, sheep and / or goats as appropriate. Animals of both sexes can be used for this type of animal experiment unless the study has to be performed in pregnant and / or lactating animals.

Purchase of animals: The animals will be purchased from commercial suppliers, obtained from affiliated farms or bred at [redacted] facilities.
If a certain microbial status is required, animals will be purchased from farms with the respective (certified) microbiological status and / or screened prior to inclusion in the study.
If the study design requires that the animals are vaccinated and / or infected at the [redacted] or when only a [redacted], the young animals have to be [redacted] accordingly from the farm of birth to the testing facility.

Special requirements
If young animals with a specific [redacted] status are required, it is often necessary to warrant that [redacted]. As these [redacted] animals are more susceptible for intercurrent infections, they [redacted] treatment. With the exception of studies against [redacted] pathogens, [redacted] can be given to the animals from [redacted] after birth onwards as an aid in the prevention of [redacted]

Age of animals:
Age of the animals for vaccine research varies from a few hours old to adult. The age of the animals to

be used should be the minimal age recommended for use of the vaccine.
If very young lambs or kids have to be vaccinated, it might be necessary to include the ewes / goats to foster the lambs / kids. For vaccines intended to be used for pregnant / lactating animals, it may be necessary to include dams in one or more specific trimester of pregnancy, depending on the vaccination schedule to be recommended. Samples might have to been taken from the offspring, for certain studies, euthanasia of the offspring might be required.

The tables below, specify, which models would be used for the different pathogens that might be included in research projects over the next 5 years. The lists are more extensive than the actual portfolio will be, but it is not possible at this moment to predict, which pathogens will be worked on.
The animal categories listed are the age groups considered to be most sensitive and therefore have to be used to perform the basic efficacy studies.

Priorities within the R&D portfolio are based on market needs and the estimated likelihood of success of obtaining a vaccine candidate that fulfills the required product profile. Priorities can shift upon identification of new unmet needs in the field. For example, if a new [redacted] pathogen with substantial impact on the ruminant industry is discovered, this will be given priority over research into a second generation improved product for a [redacted] pathogen.

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 2 wk
New pathogen	Cattle <6 Months old	Severe (max 70%)[1]	Max 2 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 2 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 2 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted][1]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 50%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
New pathogen	Cattle <6 Months old	Moderate (max 100%)[2]	Max 2 wk

[1]Vaccines are given to [redacted] in order to [redacted]. This part of the study does only cause mild discomfort. The information given in the table above relates to the discomfort during challenge studies in [redacted] will be determined.
[2]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 50%)	Max 2 wk
[redacted]	Adult cows	Moderate (max 50%)	Max 2 wk
[redacted]	Adult cows	Mild	Max 2 wk
[redacted]	Adult cows	Mild	Max 2 wk
[redacted]	Adult ewes	Mild	Max 2 wk
[redacted]	Adult ewes	Mild	Max 2 wk
New pathogen	Adult cows	Moderate (max 100%)[1]	Max 2 wk
New pathogen	Adult ewes	Moderate (max 100%)[1]	Max 2 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk

[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
New pathogen	Adult cows	Severe (max 100%)[1]	Max 2 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle<6Months and adult cows	Moderate (max 50%)	Max 2 wk

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	sheep <6 Months	Severe (max 40%)	Max 1-2 d
	cattle<6 Months	Moderate (max 70%)	Max 2 wk
[redacted]	sheep <6 Months	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 70%)	Max 1-2 days
[redacted]	sheep <6 Months	Severe (max 50%)	Max 1 wk
[redacted]	cattle<6 Months	Moderate (max 70%)	Max 1 wk
[redacted]	sheep <6 Months	Mild	Max 2 wk
	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Severe (max 50%)	Max 1-2 d
[redacted]	sheep < 6 Months	Severe (max 50%)	Max 1-2 d
[redacted]	cattle<6 Months sheep < 6 Months	Mild	Max 2 wk
New pathogens and new types of models ([redacted])	cattle<6 Months sheep < 6 Months	Severe (max 100%)[2]	Max 1 w

[1] Efficacy of [redacted] vaccines in ruminants is tested by [redacted] only

[2]: Studies with potential new pathogens are given the maximum discomfort score until the severity of clinical signs has been established

Based on the experience over the last 5 years and the current R&D program and priorities, the total expected number of cattle, sheep and goat per age group and discomfort category is the following:

Species	Discomfort score*	Animals <6 Months	Adult**
Cattle	Mild***	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]
Sheep	Mild***	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]
Goat	Mild***	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]

*: Discomfort due to disease
**: Including [redacted]
***: Due to repeated procedures the overall discomfort will be moderate

In the vaccination studies, one or more vaccinated groups are compared to an unvaccinated control group. The group size is dependent on the challenge model and the age of the animal at the time of challenge, as for some pathogens the susceptibility for infection/disease will diminish with age, making the use of relatively large group sizes necessary to be able to show a statistically significant difference. In general, the group size used will be 5-8 animals, but could be larger depending on the expected variation in the infection model.
The expected numbers per category of vaccine are:

Per Model	Cattle <6Months	Adult cattle	Sheep <6Months	Adult sheep	Goat <6Months	Adult goat
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

For the vaccines that are currently in the research phase, challenge will still have to be performed in [redacted] of the models to prove efficacy because of the absence of an immunological correlate of protection.

C. Re-use

Will the animals be re-used?

☐ No, continue with question D.

X Yes > Explain why re-use is considered acceptable for this animal procedure.

Re-use might be considered for animals that experience only minor discomfort during the first study, for example uninfected control animals. This approach is considered acceptable especially in case of animals that were raised under special conditions (e.g. [redacted]) to achieve a certain [redacted] status.

Are the previous or proposed animal procedures classified as 'severe'?

xNo

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:
In accordance with international regulations, animals of the target species must be used to demonstrate the safety and efficacy of a vaccine because there are no suitable alternatives or models for the induction of immunity in a whole organism or for the infection of living tissues as complex as those found in the whole animal in which the vaccines are intended to have efficacy. Therefore, during the research phase, candidate vaccines have to be tested in the same models.

Reduction: All studies are performed with the lowest possible number of animals that are required to enable meaningful interpretation of the results. This will be achieved through an ongoing evaluation of the observations in each study. The number of animals per study will be substantiated in each study protocol. According to internal procedures, the study protocol will be reviewed by the Animal Welfare Body and a statistician.

Refinement:
Where possible it is pursued to refine the routes of administration of substances and sampling techniques to improve animal welfare/to reduce discomfort of administration, but without endangering the scientific outcome. Ruminants are the target species and there are no other less innervated/sentient species that could be a model for the ruminant diseases that are studied. See next paragraph for other refinement methods that are applied.
The classic method to prove protection of a new vaccine is efficacy in a vaccination-challenge test. However, if immunological correlates of protection (e.g. a serological response) can be used to prove efficacy this will be used rather than challenge infection. When an infection model has to be used, humane endpoints will be employed and staff will be fully trained to recognize animals that experience discomfort. Animals will be closely monitored and additional health checks are performed to ensure that no animal is left suffering.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

For monitoring of the clinical health status of animals, all study animals will be checked at least once a day by a certified person. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.
Furthermore efforts are made to optimally enrich the environment during containment.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

The vaccines in the companys R&D program are unique and proprietary to the company. To show that vaccines are compatible (combined or associated use), a number of the safety and efficacy studies done with the individual products has to be repeated with the vaccines administered together according to international regulations and guidelines.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[] No

X Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

The animals are housed socially, but animals might have to be housed (temporarily) individually without physical contact (but in the same holding room) in order to [redacted]. Some studies may require limited bedding during containment. In a few cases, it is necessary to use no bedding because of scientific reasons.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

XYes > Will anaesthesia, analgesia or other pain relieving methods be used?

[] No > Justify why pain relieving methods will not be used.

Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Vaccination, injections (for application of challenge material, injection with [redacted] drugs or agents) as well as sampling of blood are part of normal farm practice/veterinary care and will induce only mild discomfort. If the sampling is repeated ([redacted]), the discomfort is considered to be moderate as a result of the stress when restrained and during handling of the animal.
All biotechnical procedures such as vaccination and blood sampling procedures have been described in Standard Operating Procedures (SOPs) and only well trained personnel will be responsible for the execution (GLP accredited procedures).

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Measurement of body temperatures and body weight, sampling urine, feaces, colostrum/milk, sampling on different mucosae ([redacted]), as well as [redacted] are part of normal farm practice/veterinary care and will induce only mild discomfort. If the sampling is repeated ([redacted] for the [redacted] and [redacted], [redacted] or [redacted] for the other procedures), the discomfort is considered to be moderate as a result of the stress during the fixation and handling of the animal.
All biotechnical procedures such as vaccination and sampling procedures have been described in Standard Operating Procedures (SOPs) and only well trained personnel will be responsible for the execution (GLP accredited procedures).
[redacted] animals are more susceptible to disease and might therefore encounter discomfort related to intercurrent diseases. Transport of the animals to the testing facility might cause transient discomfort for the duration of the transport, especially for animals that are transported [redacted].
Vaccination can cause a transient increase in rectal temperature, sometimes accompanied with a reduced

level of activity, and a transient vaccination site reaction. Systemic reactions generally disappear within 24 hours, but local reactions (that are generally painless) can persist for several days and even weeks. The safety and efficacy profile of the vaccine compositions used in the research phase is not yet determined. Therefore, it is possible that adverse events occur or that protection of the vaccinated animals is very limited.
Depending on the nature of the challenge inoculum the discomfort of the challenge can range from mild in the absence of any clinical signs (e.g. [redacted]) to moderate (e.g [redacted] and severe (e.g. [redacted]). Vaccination will result in a significant reduction of clinical abnormalities after challenge compared to the unvaccinated control group and thus to a reduction of animals with discomfort.
In case of challenge studies with pathogens that do not cause clinical disease or only very mild disease, it might be necessary to [redacted] the animals. These procedures and any effects related to the [redacted] treatment are already taken into consideration for the discomfort levels and durations listed above in the respective tables.
The clinical health status of all animals is checked at least once a day by qualified personnel. Special attention is paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.
In consultation with the veterinarian and Study Director, if treatment does not interfere with the test results, it will be decided whether to apply adequate veterinary care including analgesia to alleviate treatment related pain (for example infection studies with [redacted] pathogens or [redacted] or pain not related to the treatment. In case of severe suffering, humane endpoints are applicable. General humane endpoints are described in an SOP (e.g. the condition of the animal prevents it from eating and drinking regularly, severe loss of body weight) and test specific humane endpoints are given in each study protocol if applicable.

Explain why these effects may emerge.

These procedures may be part of the experimental design. For vaccines intended for use in young animals, the study design may require that the animals are vaccinated and / or infected at [redacted] or when only [redacted]. In these cases, the young animals have to be [redacted] accordingly from the farm of birth to the testing facility.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

All biotechnical procedures will only be performed according to standard procedures described in SOPs (GLP accredited procedures).
The number of samplings will be done in accordance with the respective guidelines or if no requirements are given, the number of samplings is reduced to a minimum number required to for a valid evaluation of results.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

To determine the efficacy of a vaccine it is necessary to challenge animals with the pathogenic organism. The severity of discomfort is depending on the nature of the pathogen (see 3.1 of the project proposal for specific clinical signs of the pathogens involved). However, the duration of severe discomfort will be limited due to the application of a humane endpoint if needed. Therefore, pathogen specific humane endpoints are formulated when discomfort is foreseen. These contain information about the clinical signs that can be expected and describe when a humane endpoint has been reached for (a combination of) the respective clinical signs. It also describes the scientific endpoint; the degree of progress of disease that is necessary to be able to draw conclusions about the effectiveness of the treatment tested. The humane endpoints are always leading. These test-specific humane endpoints need to be described in the corresponding study protocol. Each study protocol is reviewed by the AWB before execution of the study. In case it is difficult to reach a decision based on the pre-defined criteria for an endpoint the designated veterinarian is empowered to decide that a humane endpoint is applied/reached.
General humane endpoints (e.g. the condition of the animal prevents it from eating and drinking regularly, severe loss of body weight, pain) are described in an SOP. These endpoints are applicable to

all animals, irrespectively of the type of experiment.

Indicate the likely incidence.

Considering the expected number of studies with the different pathogens, the expected number of animals included in the different treatment groups (i.e. vaccinated / infected / control group) and the expected severity, at most 20 % of the animals is expected to have severe discomfort that would require euthanasia.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

For studies without challenge, discomfort will be mild to moderate depending on the number of sampling points. For vaccination-challenge studies, the type and severity of the clinical signs are depending on the type of challenge infection. Similar to natural field infections they may cause mild to severe pain, distress, suffering or even impending death. See B for an overview of the different pathogens involved, the maximal discomfort caused by the disease and the maximum number of animals expected to reach the highest discomfort category. Vaccination is expected to reduce the level of discomfort after challenge, but the non-vaccinated control group will experience the symptoms of the natural infection.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Postmortem investigation can be part of the experimental design to evaluate (histo)pathological lesions at the injection sites and or to evaluate effect of the infection on different organ systems and or to attempt re-isolation of the inoculum from tissues and organs.
In addition, animals vaccinated with a non-licensed vaccine or infected with a pathogen cannot be returned to the farm of origin or transported to another farm to prevent the spread of disease. Therefore, all animals might have to be euthanized at the end of the study or when a humane endpoint is reached. Control animals that have not been vaccinated and infected may be reused or returned to the farm of origin or transported to another farm. In case of a surrogate immunological marker (no challenge), animals housed on contract farms can remain on the farm or transported to other farms until the end of their natural/economic life.
Moreover, return of the animals to commercial farms or slaughter for human consumption is often prohibited by the current legislation on use of antibiotics.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	22100
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 3 Type of animal procedure: Research: Assay development and preparation of biomaterials

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

The potency of each inactivated vaccine batch used in development studies has to be determined to set the limits for release and to determine the stability. Traditionally, the potency of inactivated vaccines, is measured in a so-called *in vivo* potency in [redacted]. During the research phase of a vaccine project, the test protocol for the potency test is determined. In an initial study, the strenghth of the immune reponse is compared between the different animal species. In further studies, it is investigated, whether the antibody response is dose dependent and the release criteria are determined.
Apart from the replacement of experimental animals, *in vitro* potency tests are very much preferred over *in vivo* testing for multiple other reasons including costs and timelines. However, for several inactivated vaccines, *in vivo* potency tests are still necessary because it is either not possible to determine the antigen and/or adjuvant content *in vitro* or an *in vivo* batch test is mandatory under the respective legislation (e.g. Ph.Eur monograph.).
In such a batch potency test, animals are injected with one or more doses of the vaccine batch. The potency is preferably determined by measuring the antibody response. In a few cases, determination the protection against challenge infection is mandatory or necessary for scientific / technical reasons.
Biomaterials such as specific antisera or monoclonal antibodies are needed in most vaccine projects for different purposes such as the identification, characterisation and quantification of the vaccine strain, neutralizing the vaccine virus, setting up *in vitro* tests et cetera.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Two or more of the following treatments will be employed in order to fulfil the requirements for the potency studies as laid down in the Ph Eur *(in italics the frequency of the treatments).* The same

treatments are also applied for the preparation of biomaterials*:*
1. Blood sampling [redacted] to determine [redacted] parameters
2. Test substance administration [redacted]
3. Weighing [redacted]
4. Euthanasia
The duration of these procedures will only be minutes.
In case of potency tests that involve challenge infection, the following additional treatments are employed
5. Administration of challenge material [redacted]
6. Clinical observation [redacted]

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

The group sizes for potency tests that are specified in guidelines typically range between 5 and 10. For those models that are not described in specific regulations, a group size of 5-10 animals is generally accepted by regulatory authorities. If sufficient knowledge on the variation is available the group size will be calculated such that a statistically significant difference between standard and substandard vaccine batches can be made with 80% power and 95% confidence.
For the preparation of antisera the minimal number of animals that will provide the required volume will be used.
For the preparation of [redacted] mice will be used per antigen.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

For some vaccines, the species to be used for a batch potency test is prescribed in a Ph.Eur monograph.
In general, [redacted] are preferred over the ruminant target species because they are more genetically homogeneous and better microbiologically controlled.
Therefore, less variance in response and better reproducibility can be achieved, which means that the number of experimental animals can be lower than when using ruminants.
For some vaccines, the type of laboratory animals to be used for a batch potency test is prescribed in a Ph.Eur monograph.
Preparation of antisera in non-ruminant animals or murine monoclonal antibodies has the advantage, that these animals are free of antibodies against other ruminant pathogens.
Based on the experience over the last 5 years and the current R&D program and priorities, the total expected number of laboratory animals is the following:
[redacted]
[redacted] of both sexes will be used, but for [redacted] and [redacted] only females will be included in studies because of a higher risk of fighting in male animals and the relatively long time that the animals are in an experiment. With regard to the length of these experiments [redacted] it is not acceptable to use males, because they would need to be single housed.
Welfare concerns are the basis for the preferred use of female rabbits, mice and guinea pigs because they hardly fight. Data from recently executed experiments with male animals have shown that in half of the experiments there was a loss of animals because of severe fighting. This has resulted in the repetition of these experiments and therefore in the use of more animals. A loss of animals due to fighting has never occurred when female animals were used. We consider the aggression and fighting a worrying impairment of welfare. Moreover, the aggression will cause stress, which is known to have effects on the immune system.

In these studies the functioning of the immune system is crucial and variation in the immune response caused by external factors should be avoided as much as possible.

Origin [redacted]:
All animals are supplied by certified vendors accompanied by a health certificate according to FELASA recommendations. All purchased animals have a SPF status.

Origin [redacted]
Own SPF [redacted] breeding unit or commercial vendor.

Age of animals:
The required species and age (or weight) is usually designated as the most sensitive species or age for the test component in question. If such specific knowledge is not available the most practical choices are made, based on possibilities for purchase and housing conditions. Moreover, animals must be immunologically fit to be subjected to immunizations and blood samplings.

C. Re-use

Will the animals be re-used?

X No, continue with question D.

Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

XNo

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:
Apart from the replacement of experimental animals, *in vitro* potency tests are very much preferred over *in vivo* testing for multiple other reasons including costs and timelines. Therefore, multidisciplinary teams are active at the company to replace *in vivo* potency tests by *in vitro* tests such as antigenic mass assays for the potency testing of vaccines.

Reduction: The animal species that is expected to give the most discriminatory test with the smallest number of animals will be used. The number of animals per study will be substantiated in each study protocol. Each study protocol will be reviewed by the Animal Welfare Body and a statistician.

Refinement: Following the codes of practice for immunization is the basis for refinement in these animal procedures. Where possible it is pursued to refine the routes of administration of substances and sampling techniques to improve, but without endangering the scientific outcome. For example; blood sampling in rodents is done under anesthesia. See next paragraph for other refinement methods that are applied.
Where ever possible, potency testing will be based on the testing of antibodies or other correlates of protection rather than by challenge.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Following the code of practice for immunization and the code of practice for monitoring the welfare of the animals is the basis for refinement in these animal procedures.
In general animals are always housed socially, but animals might have to be (temporarily) separated due to fighting or because of veterinary concerns. Furthermore, to enhance animal welfare, species specific environmental enrichment is provided to all animals.
For monitoring of the clinical health status of animals, all study animals will be checked at least once a

day by a certified person. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

A potency test model needs to be developed for a specific vaccine. Experiences from vaccines with similar composition can be used, but eventually, the model has to be validated for the specific vaccine. Specific biomaterials have to be available for each project. Prior to the preparation of new biomaterials, the scintific literature and the company database for biomaterials available at the different research sites will be consulted in order to prevent unnecessary preparation of new biomaterials.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[] No

X Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

Some studies may require limited bedding during containment.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

XYes > Will anaesthesia, analgesia or other pain relieving methods be used?

X No > Justify why pain relieving methods will not be used.

X Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Injections (vaccination, inoculation of challenge material) and blood sampling are part of normal veterinary care / commonly used biotechnical procedures and will induce only mild discomfort or moderate discomfort of very short duration.

Anesthesia will be applied during blood sampling of [redacted]. Anesthesia will not be applied during sampling blood from [redacted]. In these species blood sampling causes only mild discomfort or moderate discomfort of very short duration. Applying anesthesia would only increase

discomfort as the animal needs to be restrained during administration.

For each species blood sampling and other biotechnical procedures have been described in Standard Operating Procedures (SOPs) (GLP accredited procedures).
As the vaccines to be tested have already been found to be safe in the target species (ruminants) it is very unlikely that they will cause adverse effects in non-target animals.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

In general, biotechnological procedures such as weighing and sampling may result in discomfort, because animals need to be fixated and especially if performed repeatedly.
Moreover, application of the vaccine can result in a transient increase in rectal temperature, sometimes accompanied with a reduced level of activity, and a transient vaccination site reaction. Systemic reactions will generally disappear within 24 hours, but local reactions (swelling redness), that are generally painless, can persist for several days and even weeks, but these local reactions do not affect normal behaviour (activity, feeding and drinking).
Depending on the nature of the challenge inoculum, the discomfort of the challenge can range from mild in the absence of any clinical signs (e.g. [redacted]) to moderate (e.g [redacted] and severe (e.g. [redacted]). Vaccination will result in a significant reduction of clinical abnormalities after challenge compared to the unvaccinated control group.
For monitoring of the clinical health status of animals, all study animals will be checked at least once a day by a certified person. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.
In case animals experience discomfort (whether or not related to the treatment), it will be decided in consultation with the veterinarian and Study Director whether to apply adequate veterinary care to alleviate unexpected pain and/or distress (if treatment does not interfere with the test results). In case of severe suffering, humane endpoints are applicable. General humane endpoints are described in an SOP (e.g. the condition of the animal prevents it from eating and drinking regularly, severe loss of body weight) and test specific humane endpoints are given in each study protocol if applicable.

Explain why these effects may emerge.

These procedures and if applicable the clinical signs caused by the challenge may be part of the experimental design.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Weighing is part of normal veterinary care and will induce only mild discomfort. Taking samples of mucosal surfaces or urine will result in mild discomfort, with the exception of repeated mucosal swabbing, which is considered to be moderate discomfort. Biotechnical procedures have been described in SOPs (GLP accredited procedures). Unless required by the applicaple regulations or scientific reasons, potency testing will be based on the testing of correlates of protection such as antibody levels rather than by challenge.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

In potency tests that require challenge with a pathogenic organism, the severity of discomfort is depending on the nature of the pathogen. However, the duration of severe discomfort will be limited due to the application of a humane endpoint if needed. Therefore, pathogen specific humane endpoints are formulated when discomfort is foreseen. These contain information about the clinical signs that can be expected and describe when a humane endpoint has been reached for (a combination of) the respective clinical signs. It also describes the scientific endpoint; the degree of progress of disease that is necessary to be able to draw conclusions about the effectiveness of the treatment tested. The humane endpoints are always leading. These test-specific humane endpoints need to be described in the corresponding study protocol. Each study protocol is reviewed by the AWB before execution of the study. In case it is difficult to reach a decision based on the pre-defined criteria for an endpoint the designated

veterinarian is empowered to decide that a humane endpoint is applied/reached.
General humane endpoints (e.g. the condition of the animal prevents it from eating and drinking regularly, severe loss of body weight, pain) are described in an SOP. These endpoints are applicable to all animals, irrespectively of the type of experiment.

Indicate the likely incidence.

As the overall majority of potency tests as well as the preparation of biomaterials do not require challenge, the incidence of severe discomfort requiring euthanasia is estimated to be less than 2 %.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Discomfort will be mild to moderate depending on the number of sampling points for those models that do not require challenge as well as the preparation of biomaterials.
Depending on the challenge organism, discomfort might be severe in a minority of models.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

For some potency models it is necessary to investigate the presence of (histo)pathological lesions and take organ and tissue samples for re-isolation of the vaccine strain.
For the preparation of antisera it is necessary to kill the animals in order to obtain the required volumes.
For preparation of monoclonal antibodies mice are killed to collect the lymphoid organs that are needed

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

14 juli 2016

7.

Format DEC-advies

Maak bij de toepassing van dit format gebruik van de Praktische Handreiking: Ethisch Toetsingskader voor proefdiergebruik. Voor voorbeelden, zie bijlage I.

Herhaling van antwoorden is niet nodig. Indien van toepassing kan verwezen worden naar een bij een eerdere vraag verstrekt antwoord.

A. Algemene gegevens over de procedure

1. Aanvraagnummer

2. Titel van het project: **Research of new ruminant vaccines**

3. Titel van de NTS: **Onderzoek naar nieuwe vaccins tegen ziektes bij herkauwers**

4. Type aanvraag: **nieuwe aanvraag projectvergunning**

5. Contactgegevens DEC:
   - naam DEC [redacted]
   - telefoonnummer contactpersoon [redacted]
   - e-mailadres contactpersoon [redacted]

6. Adviestraject (data dd-mm-jjjj):
   ontvangen door DEC 3 april 2017
   aanvraag compleet
   in vergadering besproken 13 april 2017
   anderszins behandeld
   termijnonderbreking(en) van / tot
   besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   aanpassing aanvraag
   advies aan CCD

7. Geef aan of de aanvraag is afgestemd met de IvD en deze de instemming heeft van de IvD. **Ja**

Bij de punten 8 t/m 10 kan worden volstaan met 'n.v.t.' wanneer de betreffende acties niet aan de orde zijn geweest.

8. Eventueel horen van aanvrager **N.v.t.**
- Datum
- Plaats
- Aantal aanwezige DEC-leden
- Aanwezige (namens) aanvrager
- Gestelde vraag / vragen
- Verstrekt(e) antwoord(en)
- Het horen van de aanvrager heeft wel/niet geleid tot aanpassing van de aanvraag

- Correspondentie met de aanvrager
- Datum 13 april 2017

- Gestelde vraag/vragen zie onder
- Datum antwoord 14 april 2017
- Verstrekt(e) antwoord(en) zie onder

Vragen vanuit de DEC over het projectvoorstel "Research of new ruminant vaccines"

(in rood de reactie van de aanvrager)

Non-technical summary

- Is het mogelijk om in 3.1 toe te voegen dat vaccinaties er ook voor zijn om de risico's van het uitbreken van zoönoses te beperken en antibiotica resistentie te voorkomen? Ja, zal worden toegevoegd, echter worden beide punten ook al 3.2 benoemd.
- Onder 3.5 graag meer verduidelijking van de genoemde percentages van ongerief. Ter verduidelijking is de tabel waarin de percentages enkel op basis van de door de ziekte veroorzaakte ongerief bepaald is, is verwijderd. Enkel de tabel waarin ook met de door de handelingen veroorzaakte ongerief rekening gehouden werd zal worden weergegeven.

Appendix 1 (sommige punten gelden ook voor andere Appendices)

- Graag opheldering gebruik [redacted] dieren die geëuthanaseerd worden om de [redacted] te verkrijgen. Zijn de [redacted] meegeteld in het totale [redacted] Over het algemeen zullen alleen dieren gedurende [redacted] worden geëuthanaseerd. Indien euthanasie [redacted] nodig is zullen [redacted] worden meegeteld in het totale [redacted]
- Op pagina 5: kan de voetnoot bij de tabel [redacted]" aangepast worden? Lijkt nu ook op mild te slaan en niet alleen op new pathogens? Wordt aangepast.
- Op pagina 7, in de tabel boven sectie C: Wat wordt er precies bedoeld met [redacted]? Voor deze studies zijn de modellen [redacted] van toepassing. De naam van de tabel zal worden gewijzigd naar [redacted]
- Gebruik analgesie (pagina 9); kan er een motivatie worden gegeven? Zijn er voorbeelden beschikbaar? De tekst zal worden aangepast: In consultation with the veterinarian and Study Director, if treatment does not interfere with the test results, it will be decided whether to apply adequate veterinary care including analgesia to alleviate treatment related pain [redacted] or pain not related to the treatment."
- Op pagina 9: er wordt over [redacted] en [redacted] samples gesproken maar die zijn nog niet eerder in de tekst genoemd Zullen worden toegevoegd aan de lijst met procedures in sectie A.
- Op pagina 10 onder J: Kan er inzage gegeven worden hoe de incidence of discomfort op 30% geschat is? Bij de schatting is rekening gehouden met het verwachte aantal studies met een bepaald pathogeen, het verwachte aantal dieren dat niet gevaccineerd, maar wel besmet zou worden en het te verwachten ongerief. Deze overwegingen zullen als volgt worden weergegeven in de betreffende [redacted] Considering the expected number of studies with the different pathogens, the expected number of animals included in the different treatment groups (i.e. treated vs control group) and the expected severity, at most XX % of the animals is expected to have severe discomfort that would require euthanasia.
- Op pagina 11 onder L: wordt er naast inspectie van de injection site ook niet naar andere organen gekeken om een beeld van het infectieproces te krijgen? Uiteraard. De tekst zal worden aangepast om dit te verduidelijken.

Appendix 2

- Op pagina 4: Kan er een verklaring gegeven worden voor de genoemde [redacted] ratio? Bedoelt wordt dat [redacted] van de projecten betrekking hebben op [redacted] vaccins en in [redacted] van de projecten [redacted] vaccins [redacted] worden. Echter is de ratio niet direkt te projecteren op het aantal studies of het aantal dieren. Daarom lijkt het bij nader inzien beter, de alinea geheel te verwijderen.
- Op pagina 6: wat wordt er precies met de [redacted] bedoeld in de tabel? Naar aanleiding van de vraag van de DEC zijn we tot de conclusie gekomen, dat dit onderzoek niet direct binnen de doelstelling "verbetering van gezondheid bij dier / mens" valt. Er zijn op dit moment geen concrete plannen voor dit onderzoek. Daarom zullen verwijzingen naar dit onderzoek uit de tabel en het project voorstel verwijderd worden.
- Op pagina 9: onder I is het nodig om analgesie te noemen en voorbeelden (zie appendix 1) Tekst zal worden aangepast zoals voor appendix 1.
- Op pagina 10 onder J: Kan er inzage gegeven worden hoe de "incidence of discomfort" op 20% geschat is? Zie appendix 1.

Appendix 3

- Op pagina 4 onder F: worden ook [redacted] en [redacted] individueel gehuisvest? Nee. De betreffende zin zal worden verwijderd.
- Op pagina 6 onder L: is het voor de ontwikkeling van [redacted] beter om van [redacted] organen te spreken i.p.v. alleen de [redacted] Tekst zal worden aangepast om ook het ontnemen van andere [redacted] organen mogelijk te maken.

- De antwoorden hebben **wel** geleid tot aanpassing van de aanvraag

9. Eventuele adviezen door experts (niet lid van de DEC)
   - Aard expertise
   - Deskundigheid expert
   - Datum verzoek
   - Strekking van het verzoek
   - Datum expert advies
   - Advies expert

B. Beoordeling (adviesvraag en behandeling)

1. Is het project vergunningplichtig (dierproeven in de zin der wet)? **Ja**
2. De aanvraag betreft een **nieuwe aanvraag**.

3. Is de DEC competent om hierover te adviseren? **Ja**

4. Geef aan of DEC-leden, met het oog op onafhankelijkheid en onpartijdigheid, zijn uitgesloten van de behandeling van de aanvraag en het opstellen van het advies. Indien van toepassing, licht toe waarom. **Er zijn geen DEC leden uitgesloten van de behandeling van de aanvraag en het opstellen van het advies**

C. Beoordeling (inhoud)

1. Beoordeel of de aanvraag toetsbaar is en voldoende samenhang heeft *(Zie handreiking 'Invulling definitie project'; zie bijlage I voor toelichting en voorbeeld).* **Deze aanvraag heeft een concrete doelstelling: "Research of new ruminant vaccines". Het project heeft betrekking op dierstudies met nieuwe en verbeterde vaccins in de "research fase". In de "research fase" worden**

nieuwe ziekteverwekkers geïdentificeerd, wordt de kennis van bekende ziekteverwekkers uitgebreid en wordt onderzoek gedaan naar de verbetering van bestaande vaccins, bijvoorbeeld door meerder vaccins te combineren in één product of door nieuwe formuleringen voor bestaande vaccins te ontwerpen. Deze fase gaat vooraf aan de "development fase", waarin de wettelijk verplichte experimenten worden gedaan die nodig zijn om het registratiedossier op te bouwen. In de "research fase" wordt al geanticipeerd op de "development fase" door bij de experimenten veel aandacht te schenken aan de vraag of de te ontwikkelen vaccins in een latere fase zullen kunnen voldoen aan de wettelijke eisen.
Ongeacht de vraag om welke ziekteverwekker het gaat wordt in deze fase een min of meer vaste strategie gevolgd die achtereenvolgens bestaat uit infectiestudies (is het geïsoleerde micro-organisme of producten daarvan de veroorzaker van de aandoening?), het opzetten van een infectiemodel, het ontwerpen en vervolmaken van een vaccinformulering (o.a. met behulp van immunogeniteitsstudies en met het infectiemodel) en het uitvoeren van oriënterende veiligheids- en werkzaamheidsstudies met een kandidaatvaccin. Waar mogelijk en nodig worden experimenten uitgevoerd volgens de richtlijnen van de regelgevende autoriteiten (o.a. de EP). Ook de infectiemodellen die in deze fase worden opgezet, en die zullen worden gebruikt voor de wettelijk verplichte werkzaamheidsstudies, dienen te voldoen aan bepaalde randvoorwaarden.
De beschreven dierproeven zijn duidelijk wat betreft de uit te voeren handelingen en daarmee gepaard gaande ongerief voor het individuele dier. In de overgrote meerderheid van de gevallen zijn het de experimentele procedures, en niet de aard van de ziekteverwekker of het antigeen, die de mate van welzijnsaantasting van de proefdieren bepalen (zie C11). De eenvormigheid qua design en het routinematige karakter van de dierexperimenten, is volgens de DEC één van de redenen waarom dit project als een toetsbare eenheid kan worden beschouwd.

2. Signaleer of er mogelijk tegenstrijdige wetgeving is die het uitvoeren van de proef in de weg zou kunnen staan. Het gaat hier om wetgeving die gericht is op de gezondheid en welzijn van het dier of het voortbestaan van de soort (bijvoorbeeld Wet dieren en Flora- en faunawet).
Voor zover bekend bij de DEC zijn er geen aspecten in de aanvraag die niet in overeenstemming zijn met andere wet- en regelgeving.

3. Beoordeel of de in de projectaanvraag aangekruiste doelcategorie(ën) aansluit(en) bij de hoofddoelstelling. Nevendoelstellingen van beperkt belang hoeven niet te worden aangekruist in het projectvoorstel. **De DEC is van mening dat de genoemde doelcategorie "translationeel" aansluit bij de hoofddoelstelling. In deze fase is nog geen sprake van wettelijk verplicht onderzoek, al realiseert men zich bij het design van de experimenten in deze fase al wel terdege dat in een volgende fase wettelijk verplicht onderzoek zal moeten worden gedaan.**

Belangen en waarden

4. Benoem zowel het directe doel als het uiteindelijke doel en geef aan of er een directe en reële relatie is tussen beide doelstellingen. Beoordeel of het directe doel gerechtvaardigd is binnen de context van het onderzoeksveld *(Zie Praktische handreiking ETK: Stap 1.C4; zie bijlage I voor voorbeeld).).* **Het directe doel van het project is het ontwerpen van nieuwe en verbeterde vaccins tegen infectieziekten bij herkauwers en oriënterende studies uitvoeren met deze vaccins met het oog op de hierna volgende development fase. Het**

uiteindelijke doel is het reduceren of voorkomen van infecties bij herkauwers en bij zoonotische micro-organismen door nieuwe of verbeterde vaccins op de markt te brengen. Er is een reële relatie tussen het directe doel en het uiteindelijke doel. Het directe doel is gerechtvaardigd binnen de context van het onderzoeksveld.

5. Benoem de belanghebbenden in het project en beschrijf voor elk van de belanghebbenden welke morele waarden in het geding zijn of bevorderd worden *(Zie Praktische handreiking ETK: Stap 2.B en tabel 1; zie bijlage I voor voorbeeld)* **De belanghebbenden in dit onderzoeksproject zijn de proefdieren, de aanvrager en de doeldieren (herkauwers), hun eigenaren en de maatschappij in het algemeen. De proefdieren in het project zullen verschillende niveaus van ongerief ondergaan, waardoor hun welzijn wordt aangetast. De uiteindelijke doeldieren (herkauwers) zal veel stress en ongerief bespaard blijven, omdat de vaccins bescherming tegen infecties zullen bieden. De aanvrager heeft een aanzienlijk economisch belang bij het ontwerpen van nieuwe en verbeterde vaccins voor herkauwers teneinde zijn bestaande product portfolio uit te breiden en te verbeteren. Ook de veehouders die hun dieren laten vaccineren hebben een aanzienlijk economisch belang, maar zeker ook een belang vanuit hun zorg voor de dieren, bij het beschikbaar komen van goede (combinatie)vaccins die tijdens zo min mogelijk vaccinatiemomenten kunnen worden toegediend. De samenleving en de burgers hebben een direct belang bij het terugdringen van het gebruik van antibiotica in de veehouderij, iets waar vaccins aan bijdragen. Een aantal van de vaccins beschermt de dieren tegen zoönoses die ook voor de mens een bedreiging vormen.**

6. Geef aan of er sprake kan zijn van substantiële milieueffecten. Zo ja, benoem deze, leg uit waarom daar sprake van kan zijn en geef aan of deze effecten afgedekt worden door specifieke wet- en regelgeving op het gebied van het omgaan met voor het milieu risicovolle stoffen of organismen. **Er is geen sprake van substantiële milieueffecten. Bij het onderzoek met ziekteverwekkende micro-organismen wordt gewerkt in overeenstemming met de geldende wet- en regelgeving voor inperking van deze organismen. Dieren die gevaccineerd zijn met niet geregistreerde vaccins of die gechallenged zijn met ziekteverwekkers worden, op grond van bestaande wettelijke regels, gedood om te voorkomen dat producten van die dieren (zoals vlees en melk) in de voedselketen terecht komen.**

Proefopzet en haalbaarheid

7. Beoordeel of de kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven voldoende gewaarborgd zijn. Licht uw beoordeling toe. *(Zie Praktische handreiking ETK: Stap 1.C5).* **De DEC is ervan overtuigd dat de aanvrager over voldoende expertise en infrastructuur beschikt om de doelstelling van het onderzoek binnen de gevraagde termijn te realiseren. Dit wordt ondersteund door het feit dat de aanvrager al eerder succesvol vaccins voor herkauwers heeft ontwikkeld.**

8. Beoordeel of het project goed is opgezet, de voorgestelde experimentele opzet en uitkomstparameters logisch en helder aansluiten bij de aangegeven doelstellingen en of de gekozen strategie en experimentele aanpak kan leiden tot het behalen van de doelstelling binnen het kader van het project. Licht uw beoordeling toe. *Zie Praktische handreiking ETK: Stap 1.C6)*. **De DEC is van mening de gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. Het onderzoek wordt voor het grootste deel, maar niet alleen, uitgevoerd in de doeldiersoorten, met**

uitzondering van de dierproeven in bijlage 3 waar in andere diersoorten testen worden ontwikkeld en biomaterialen die nodig zijn voor het onderzoek worden verzameld. Uitvoering van het onderzoek in het doeldier waarborgt directe toepasbaarheid van de resultaten. Het opzetten van infectiemodellen met in het veld aangetroffen nieuwe (varianten van) ziekteverwekkers en het uitvoeren van oriënterende veiligheids- en effectiviteitsstudies vindt steeds plaats volgens vaste en beproefde procedures. Het behalen van de directe doelstellingen is vooral afhankelijk van het strikt volgen van die beproefde procedures. De commissie heeft op grond van eerdere ervaring met toetsing van het onderzoek van de aanvrager er vertrouwen in dat er desondanks ruimte is om nieuwe inzichten op het gebied van de drie V's door te voeren in het onderzoek. De aanvrager heeft zeer veel ervaring met dit type onderzoek.

Welzijn dieren

9. Geef aan of er sprake is van één of meerdere bijzondere categorieën van dieren, omstandigheden of behandeling van de dieren. Beoordeel of de keuze hiervoor voldoende wetenschappelijk is onderbouwd en of de aanvrager voldoet aan de in de Wet op de Dierproeven (Wod). voor de desbetreffende categorie genoemde beperkende voorwaarden. Licht uw beoordeling toe *(Zie Praktische handreiking ETK: Stap 1.C1; zie bijlage I voor toelichting en voorbeelden).*

☐ Bedreigde diersoort(en) (10e, lid 4)
☐ Niet-menselijke primaten (10e)
☐ Dieren in/uit het wild (10f)
x Niet gefokt voor dierproeven (11, bijlage I richtlijn)
☐ Zwerfdieren (10h)
x Hergebruik (1e, lid 2)
☐ Locatie: buiten instelling vergunninghouder (10g)
☐ Geen toepassing verdoving/pijnbestrijding (13)
☐ Dodingsmethode niet volgens bijlage IV richtlijn (13c, lid 3)

Voor dit onderzoek is het gebruik van de uiteindelijke doeldiersoorten (runderen, geiten, schapen) onvermijdelijk. Het is gebruikelijk daarvoor dieren aan te kopen die niet speciaal voor dierproeven zijn gefokt.

10. Geef aan of de dieren gehuisvest en verzorgd worden op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU. Indien niet aan deze minimale eisen kan worden voldaan, omdat het, om redenen van dierenwelzijn of diergezondheid of om wetenschappelijke redenen, noodzakelijk is hiervan af te wijken, beoordeel of dit in voldoende mate is onderbouwd. Licht uw beoordeling toe. **De te gebruiken dieren worden in principe gehuisvest en verzorgd op een wijze die voldoet aan de eisen gesteld in bijlage III van de richtlijn 2010/63/EU. In een aantal gevallen is (tijdelijk) individuele huisvesting noodzakelijk om overdracht van ziekteverwekkers op andere dieren te voorkomen. Ook is in sommige gevallen om wetenschappelijk redenen niet mogelijk om de dieren bedding te geven. Dit komt overeen met wat in dit type onderzoek (infectiestudies) gebruikelijk is en is voldoende onderbouwd.**

11. Beoordeel of het cumulatieve ongerief als gevolg van de dierproeven voor elk dier realistisch is ingeschat en geclassificeerd. Licht uw beoordeling toe *(Zie Praktische handreiking ETK: Stap 1.C2)*. **Het ongerief van de dierproeven is realistisch ingeschat en geclassificeerd. De dieren in de voorgestelde experimenten worden onderworpen aan infectiestudies en vaccinatiestudies waarin wordt gekeken naar immunogeniteit, veiligheid en werkzaamheid (challenges). In een zeer groot deel van de gevallen leidt dat tot licht of matig ongerief (respectievelijk 48% en 43%), dat vooral bepaald wordt door de bekende**

routinematige procedures die deel uitmaken van deze experimenten: toediening van het vaccin, monsternames, toediening van de ziekteverwekker en observatie van symptomen. In een veel kleiner deel van

12. de gevallen is het nodig om de dieren in het kader van werkzaamheidsstudies te challengen met ziekteverwekkers die ernstige ziekteverschijnselen kunnen veroorzaken. Daarbij dient te worden aangetekend dat veel van de dieren die met deze ziekteverwekkers worden gechallenged beschermd zullen zijn door het te testen vaccin. Een ernstige aantasting van het welzijn zal zich naar verwachting alleen voordoen in onbeschermde controledieren of dieren die door een lage dosis van het vaccin niet volledig beschermd zijn (maximaal 9% van het totaal aantal dieren in de aanvraag). De aard van deze verschijnselen is voor alle gangbare ziekteverwekkers bekend en in de loop van vele jaren zijn bij de aanvrager in nauwe samenspraak tussen onderzoekers, proefdierdeskundigen/IvD en de DEC strikte criteria voor humane eindpunten ontwikkeld. Voor alle dieren in de projectaanvraag geldt dat navolgbaar is in welk soort experiment zij zullen worden ingezet, welke handelingen ze zullen ondergaan en welke gevolgen dat heeft voor hun welzijn.
De kans dat er binnen de looptijd van dit project nieuwe ziekteverwekkers zullen worden aangetroffen is reëel, maar niet heel groot. Aangezien niet vooraf bekend is hoeveel ongerief de infectiemodellen voor deze nieuwe ziekteverwekkers zullen veroorzaken, is voor de zekerheid uitgegaan van ernstig ongerief. Het betreft een beperkt aantal dieren van het totaal en gezien het belang van het zo snel mogelijk indammen van nieuwe infectieziekten, acht de DEC een beperkte mate van onzekerheid over ernst en aard van het ongerief aanvaardbaar.

13. Het uitvoeren van dierproeven zal naast het ongerief vaak gepaard gaan met aantasting van de integriteit van het dier. Beschrijf op welke wijze er sprake is van aantasting van integriteit. *(Zie Praktische handreiking ETK: Stap 1.C2). (zie bijlage I voor voorbeeld).* **Elke dierproef vormt, door de vrijheidsbeperking en de aantasting van de lichamelijke integriteit voor instrumentele doeleinden, een aantasting van de integriteit van het dier. Het toedienen van vaccins, het afnemen van bloed en het toedienen van ziekteverwekkers en de gevolgen daarvan, kunnen natuurlijk beschouwd worden als een aantasting van de integriteit van de dieren (opzettelijk ziek maken), maar de DEC is van oordeel dat bij deze handelingen het ongerief (de welzijnsaantasting) op de voorgrond staat. De aantasting van de integriteit van de dieren is daarmee vergeleken beperkt.**

14. Beoordeel of de criteria voor humane eindpunten goed zijn gedefinieerd en of goed is ingeschat welk percentage dieren naar verwachting een humaan eindpunt zal bereiken. Licht uw beoordeling toe *(Zie Praktische handreiking ETK: Stap 1.C3)*. **De aard van de ziekteverschijnselen is voor alle gangbare ziekteverwekkers bekend en in de loop van vele jaren zijn bij [redacted] in nauwe samenspraak tussen onderzoekers, proefdierdeskundigen/IvD en de DEC strikte criteria voor humane eindpunten ontwikkeld. Voor elk werkprotocol worden de humane eindpunten en de eindverantwoordelijkheid voor het toepassen daarvan, tot in detail afgestemd met de IvD.**

3V's

15. Beoordeel of de aanvrager voldoende aannemelijk heeft gemaakt dat er geen geschikte vervangingsalternatieven zijn. Licht uw beoordeling toe *(Zie Praktische handreiking ETK: Stap 1.C3)*. **Er zijn (nog) geen methoden die de voorgestelde dierproeven geheel of gedeeltelijk zouden kunnen vervangen. Herkauwers**

als runderen en schapen zijn in veel van de experimenten zowel proefdier als doeldier. Bovendien hebben de voorgestelde veiligheids- en werkzaamheidsstudies een sterk routinematig karakter. Bij de opzet en uitvoering wordt vaak noodzakelijkerwijs vooruitgelopen op de wettelijk verplichte experimenten die moeten worden verricht in het kader van de registratie van de vaccins. Van die laatste experimenten is het design in veel gevallen tot in detail door de autoriteiten voorgeschreven of met de autoriteiten afgesproken. Er is er (nog) geen zinvol vervangingsalternatief voor deze experimenten. Daar waar mogelijk wordt gebruik gemaakt van *in vitro* experimenten.

16. Beoordeel of het aantal te gebruiken dieren realistisch is ingeschat en of er een heldere strategie is om ervoor te zorgen dat tijdens het project met zo min mogelijk dieren wordt gewerkt waarmee een betrouwbaar resultaat kan worden verkregen. Licht uw beoordeling toe *(Zie Praktische handreiking ETK: Stap 1.C3)*. *)*. **Het aantal benodigde dieren wordt bepaald door in voorbereidende experimenten een goed beeld te vormen van de spreiding in de respons van de individuele dieren op het vaccin en/of de ziekteverwekker. Met behulp van die gegevens kan op basis van een statistische berekening worden bepaald welke aantallen dieren nodig zijn voor significante resultaten in vervolgonderzoek . De aanvrager heeft op basis van eerdere ervaring met dit soort experimenten een realistische inschatting gemaakt van het totaal aantal te gebruiken dieren. Het betreft voor dit onderzoeksveld gebruikelijke aantallen dieren per groep.**

17. Beoordeel of het project in overeenstemming is met de vereiste van verfijning van dierproeven en het project zodanig is opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd. Licht uw beoordeling toe *(Zie Praktische handreiking ETK: Stap 1.C3)*. **De aard van de verwachte ziekte symptomen is voor het merendeel van de ziekteverwekkers bekend en er zijn strikte criteria voor humane eindpunten ontwikkeld. Verder zullen er reagentia ontwikkeld worden om immunologische en serologische assays voor antigeen detectie en -kwantificering op te zetten. Tevens zal onderzocht worden of deze reagentia ook gebruikt kunnen worden om de challenge experimenten om te kunnen zetten in experimenten waarin op basis van analyse van bloedmonsters de werkzaamheid van een vaccin kan worden bepaald. Het project is in overeenstemming met de vereiste van verfijning van dierproeven en is zo opgezet dat de dierproeven zo humaan mogelijk worden uitgevoerd.**

18. Beoordeel, indien het wettelijk vereist onderzoek betreft, of voldoende aannemelijk is gemaakt dat er geen duplicatie plaats zal vinden en of de aanvrager beschikt over voldoende expertise en informatie om tijdens de uitvoering van het project te voorkomen dat onnodige duplicatie plaatsvindt. Licht uw beoordeling toe. **Het betreft unieke door de aanvrager te ontwikkelen vaccins. De aanvrager zal in alle fasen van het onderzoek de proeven zo ontwerpen dat ze voldoen aan wettelijke eisen voor markttoelating.**

Dieren in voorraad gedood en bestemming dieren na afloop proef

19. Geef aan of dieren van beide geslachten in gelijke mate ingezet zullen worden. Indien alleen dieren van één geslacht gebruikt worden, beoordeel of de aanvrager dat in voldoende mate wetenschappelijk heeft onderbouwd. *(Zie Praktische handreiking ETK: Stap 1.C3; zie bijlage I voor voorbeeld).* **Binnen dit project zullen in principe dieren van beide geslachten in de experimenten gebruikt worden. Daar waar**

richtlijnen het gebruik van een bepaald geslacht voorschrijven zullen de betreffende richtlijnen gevolgd worden. De DEC is van oordeel dat het voor de hand ligt dat vaccins die bijvoorbeeld bedoeld zijn voor dieren die lacteren of drachtig zijn, getest worden in de vrouwelijke doeldieren. In het geval van [redacted] en [redacted] verdient het (op grond van eerdere ervaringen) de voorkeur om alleen met vrouwelijke dieren te werken om vechten bij mannelijke dieren te voorkomen. De mannelijke dieren solitair huisvesten, zodra het vechten zich voordoet, is geen acceptabele oplossing, omdat het vaak langdurige experimenten betreft. Ook moeten alle dieren dan solitair gehuisvest worden om te voorkomen dat de spreiding toeneemt. Vechten kan verder leiden tot extra uitval van dieren en mislukken van de experimenten.

20. Geef aan of dieren gedood worden in kader van het project (tijdens of na afloop van de dierproef). Indien dieren gedood worden, geef aan of en waarom dit noodzakelijk is voor het behalen van de doelstellingen van het project. Indien dieren gedood worden, geef aan of er een voor de diersoort passende dodingsmethode gebruikt wordt die vermeld staat in bijlage IV van richtlijn 2010/63/EU. Zo niet, beoordeel of dit in voldoende mate is onderbouwd. Licht uw beoordeling toe. Indien van toepassing, geeft ook aan of er door de aanvrager ontheffing is aangevraagd *(Zie Praktische handreiking ETK: Stap 1.C3).* **In het project zullen een aantal (maar niet alle) dieren worden gedood aan het einde van het experiment. De DEC is er van overtuigd dat dit alleen gebeurt als het voor de doelstelling noodzakelijk is om na afloop van de proef weefsels te isoleren of als de dieren met ziekteverwekkers of niet geregistreerde vaccins behandeld zijn en mogelijk een gevaar vormen voor hun omgeving of het milieu. De aanvrager gebruikt methoden die beschreven zijn in bijlage IV van de richtlijn 2010/63/EU.**

21. Indien niet-humane primaten, honden, katten of landbouwhuisdieren worden gedood om niet-wetenschappelijke redenen, is herplaatsing of hergebruik overwogen? Licht toe waarom dit wel/niet mogelijk is. **In alle gevallen waarin het niet noodzakelijk is om de gebruikte landbouwhuisdieren te doden (zie criteria onder C19), wordt de mogelijkheid van hergebruik actief onderzocht. In veel gevallen wordt hergebruik ook gerealiseerd.**

NTS

22. Is de niet-technische samenvatting een evenwichtige weergave van het project en begrijpelijk geformuleerd? **De niet-technische samenvatting is een evenwichtige weergave van het project en is duidelijk geformuleerd.**

D. Ethische afweging

1. Benoem de centrale morele vraag *(Zie Praktische handreiking ETK: Stap 3.A).* **Rechtvaardigt het belang van de doelstelling van het project het ongerief dat de dieren wordt aangedaan, en is aan alle zorgvuldigheidseisen (3V's) voldaan?**

2. Weeg voor de verschillende belanghebbenden, zoals beschreven onder C5, de sociale en morele waarden waaraan tegemoet gekomen wordt of die juist in het geding zijn, ten opzichte van elkaar af. Om dit proces te vergemakkelijken, kunt u de belangrijkste

belanghebbenden en de belangrijkste waarden die in het geding zijn waarderen. U kunt dit verwoorden in termen van gering, matig of veel/ernstig voordeel of nadeel. Geef aan waarom de DEC bevordering van waarden (baten) voor de ene belanghebbende prevaleert boven de aantasting van waarden (kosten) voor de andere belanghebbende *(Zie Praktische handreiking ETK: Stap 3.B; zie bijlage I voor voorbeelden).*

Voor het merendeel van de dieren (91%) die gebruikt worden in de voorgestelde experimenten leiden de experimenten tot licht of matig ongerief en een beperkte aantasting van hun integriteit. Als de werkzaamheid van de vaccins wordt getest kan dit voor een beperkt deel van de dieren (9% van het totaal) leiden tot ernstig ongerief, omdat in de infectieproeven en challengeproeven niet gevaccineerde (controle)dieren moeten worden meegenomen. De onderzoekers doen de challengeproeven slechts als er geen alternatief voorhanden is. De duur en de ernst van het ongerief worden door de onderzoekers zoveel mogelijk beperkt.
Daar staat tegenover dat beschikbaar komen van nieuwe of verbeterde vaccins tegen infectieziekten zal bijdragen aan het verminderen van de kans op het uitbreken van infectieziekten in de doeldieren. Dit bespaart grote aantallen dieren veel leed.
De aanvrager heeft een groot economisch belang bij het op de markt kunnen brengen van nieuwe en verbeterde vaccins. Dierproeven in de doeldiersoorten zijn nodig voor het onderzoek naar de eigenschappen van de ziekteverwekkers en naar voor het ontwikkelen van vaccins.
De houders en eigenaren van de dieren hebben eveneens een groot economisch belang bij het beschikbaar komen van goede vaccins tegen een gunstige prijs. Bij een ziekte-uitbraak op hun bedrijf leiden ze grote economische en immateriële schade. Als zij hun dieren goed kunnen beschermen verbetert dat hun concurrentiepositie. Ook hebben veehouders vanuit hun zorg voor de dieren belang bij het beschikbaar komen van goede vaccins.
Ziekte-uitbraken bij herkauwers kunnen in een samenleving waarin het houden van runderen en schapen voor de productie van vlees en zuivel een grootschalige economische activiteit is, tot ernstige ontwrichting van die samenleving leiden en voor grote economische schade zorgen, ook buiten de veehouderij. Het kunnen beschikken over goede vaccins is een substantieel belang. De aanvrager draagt daar met dit onderzoek aan bij.
Tot slot dragen vaccins bij aan een beperking van het gebruik van antibiotica in de veehouderij en, voorzover de vaccins bescherming bieden tegen zoönoses, beschermen ze ook mensen tegen het oplopen van zoönoses. Het maatschappelijk belang daarvan is groot.
De DEC acht de economische belangen van de aanvrager en van de dierhouders op zich legitiem en zij leggen zeker enig gewicht in de schaal, maar alleen in combinatie met het grote maatschappelijk belang en de voordelen voor de doeldieren, namelijk betere vaccins en minder vaccinatiemomenten, rechtvaardigen ze het gebruik van de dieren in de experimenten.

3. Beantwoord de centrale morele vraag. Maak voor het beantwoorden van deze vraag gebruik van bovenstaande afweging van morele waarden. Maak daarnaast gebruik van de volgende moreel relevante feiten: belang onderzoek (C4), kennis en kunde van betrokkenen (C7), haalbaarheid doelstellingen (C8), categorieën en herkomst dieren (C9), 3V's (C14-C18), ongerief (C10-13 en C19) en relevante wet en regelgeving (C2). Onderbouw hoe al deze elementen zijn meegewogen bij de beantwoording van de centrale morele vraag, zodanig dat het navolgbaar is zonder gedetailleerde kennis te hebben van het projectvoorstel *(Zie Praktische handreiking ETK: Stap 3.C; zie bijlage I voor voorbeeld)*. **De DEC is overtuigd van het belang van de doelstellingen beschreven in het projectvoorstel "Research of new ruminant vaccines". Volgens de DEC wegen de voordelen voor de doeldieren, de samenleving, de aanvrager en de houders van de dieren gezamenlijk zwaarder**

dan de nadelen voor de gebruikte proefdieren. Het project is goed opgezet. Verder is de DEC van mening dat de aanvrager voldoende kennis en kunde heeft om te kunnen voldoen aan de 3V beginselen en dat de aanvrager ervoor zal zorgen dat het ongerief van de proefdieren zoveel mogelijk beperkt zal worden. Gelet op het bovenstaande is de DEC unaniem van oordeel dat voor het project "Research of new ruminant vaccines" het gebruik van de proefdieren gerechtvaardigd is.

E. Advies

1. Advies aan de CCD

☐ **De DEC adviseert de vergunning te verlenen.**

x De DEC adviseert de vergunning te verlenen onder de volgende voorwaarden

x Op grond van het wettelijk vereiste dient de projectleider bij beëindiging van het project een beoordeling achteraf aan te leveren die is afgestemd met de IvD.

☐ Voor de uitvoering van dit project is tevens ministeriële ontheffing vereist

☐ Overige door de DEC aan de uitvoering verbonden voorwaarden, te weten...

☐ De DEC adviseert de vergunning niet te verlenen vanwege:

☐ De vaststelling dat het project niet vergunningplichtig is om de volgende redenen:…

☐ De volgende doorslaggevende ethische bezwaren:…

☐ De volgende tekortkomingen in de aanvraag:…

2. Het uitgebrachte advies kan unaniem tot stand zijn gekomen dan wel gebaseerd zijn op een meerderheidsstandpunt in de DEC. Indien gebaseerd op een meerderheidsstandpunt, specificeer het minderheidsstandpunt op het niveau van verschillende belanghebbenden en de waarden die in het geding zijn *(Zie Praktische handreiking ETK: Stap 4.A; zie bijlage I voor voorbeeld).* **Het advies is unaniem tot stand gekomen**

3. Omschrijf de knelpunten/dilemma's die naar voren zijn gekomen tijdens het beoordelen van de aanvraag en het opstellen van het advies zowel binnen als buiten de context van het project *(Zie Praktische handreiking ETK: Stap 4.B)*.

De aanvrager stelt in de aanvraag: "One aspect is however constant across the whole ruminant health market: the trend for increasing animal productivity and growing farm/herd size, which is worsening the impact of infectious diseases". De DEC wil in dat verband opmerken dat dit advies is geschreven vanuit het perspectief van een samenleving waarin de grootschalige productie van vlees en zuivel tegen een zo laag mogelijke prijs een geaccepteerde economische activiteit is. Op de wereldwijde markt is de concurrentiedruk groot. Wanneer die economische context als een onontkoombaar gegeven wordt aangenomen, dan weegt het belang van dit onderzoek op tegen het ongerief van de dieren die er voor gebruikt worden.

Het is de unanieme mening van de DEC dat de druk om zo goedkoop mogelijk (en per dier zo veel mogelijk) te produceren tot niet optimale leefomstandigheden en relatief kwetsbare dieren leidt. Vooral bij runderen (melkvee en kalveren) is dit het geval. Een relatief kwetsbare gezondheid en niet optimale leefomstandigheden kunnen op zichzelf al bijdragen aan het ontstaan van ziekten.

De DEC zou het betreuren als het beschikbaar komen van steeds betere vaccins tegen allerlei ziekten bij herkauwers ertoe leidt dat er niets gedaan wordt om de leefomstandigheden en de robuustheid van de dieren te verbeteren. Het

beter afstemmen van de leefomstandigheden op de natuurlijke behoeften van de dieren zal weliswaar niet in alle gevallen (volledig) het uitbreken van ziekten kunnen voorkomen, en dus ook vaccins niet overbodig maken, maar het kan wel het welzijn van de dieren aanmerkelijk verbeteren. De DEC van de aanvrager zou het toejuichen wanneer dit in striktere richtlijnen wordt vastgelegd.

Centrale Commissie Dierproeven

8.

> Retouradres Postbus 20401 2500 EK Den Haag

[redacted]

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD2210020171629

Bijlagen
2

Datum 2 mei 2017
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

[redacted]

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 2 mei 2017. Het gaat om uw project "Research of new ruminant vaccines". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD2210020171629. Gebruik dit nummer wanneer u contact met de CCD opneemt.

Wacht met de uitvoering van uw project
Als wij nog informatie van u nodig hebben dan ontvangt u daarover bericht. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn bijgeschreven op de rekening van de CCD. U ontvangt binnen veertig werkdagen een beslissing op uw aanvraag. Als wij nog informatie van u nodig hebben, wordt deze termijn opgeschort. In geval van een complexe aanvraag kan deze termijn met maximaal vijftien werkdagen verlengd worden. U krijgt bericht als de beslisperiode van uw aanvraag vanwege complexiteit wordt verlengd. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

Factuur
Bijgaand treft u de factuur aan voor de betaling van de leges. Wij verzoeken u de leges zo spoedig mogelijk te voldoen, zodat we uw aanvraag in behandeling kunnen nemen. Is uw betaling niet binnen dertig dagen ontvangen, dan kan uw aanvraag buiten behandeling worden gesteld. Dit betekent dat uw aanvraag niet beoordeeld wordt en u uw project niet mag starten.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Datum:
2 mei 2017
Aanvraagnummer:
AVD2210020171629

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Datum:
2 mei 2017
Aanvraagnummer:
AVD2210020171629

Gegevens aanvrager

Uw gegevens

Deelnemersnummer NVWA:	22100
Naam instelling of organisatie:	[redacted]
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	[redacted]
Straat en huisnummer:	[redacted]
Postbus:	[redacted]
Postcode en plaats:	[redacted]
IBAN:	[redacted]
Tenaamstelling van het rekeningnummer:	[redacted]

Gegevens verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	[redacted]
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Datum:
2 mei 2017
Aanvraagnummer:
AVD2210020171629

Gegevens plaatsvervangende verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	[redacted]
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Over uw aanvraag

Wat voor aanvraag doet u?	[x] Nieuwe aanvraag [ ] Wijziging op een (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn [ ] Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn

Over uw project

Geplande startdatum:	1 september 2017
Geplande einddatum:	1 september 2022
Titel project:	Research of new ruminant vaccines
Titel niet-technische samenvatting:	Onderzoek naar nieuwe vaccins tegen ziektes bij herkauwers
Naam DEC:	[redacted]
Postadres DEC:	[redacted]
E-mailadres DEC:	[redacted]

Betaalgegevens

De leges bedragen:	€ 1.541,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

Verplichte bijlagen:	[x] Projectvoorstel [x] Beschrijving Dierproeven [x] Niet-technische samenvatting
Overige bijlagen:	[x] DEC-advies

Ondertekening

Naam: [redacted]
Functie: [redacted]
Plaats: [redacted]
Datum: 2 mei 2017

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

[redacted]

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD2210020171629

Bijlagen
2

Datum 2 mei 2017
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 2 mei 2017
Vervaldatum: 1 juni 2017
Factuurnummer: 171629

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD2210020171629	€ 1.541,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL29INGB 070.500.1512 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 93144, 2509 AC te 's Gravenhage.

Centrale Commissie Dierproeven

9.

> Retouradres Postbus 20401 2500 EK Den Haag

[redacted]

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD2210020171629

Datum 10 mei 2017
Betreft aanvraag projectvergunning Dierproeven

[redacted]

Op 2 mei 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Research of new ruminant vaccines" met aanvraagnummer AVD2210020171629. In uw aanvraag zitten voor ons nog enkele onduidelijkheden. In deze brief leest u wat wij nog nodig hebben en wanneer u een beslissing kunt verwachten.

Welke informatie nog nodig
Wij hebben de volgende informatie van u nodig om uw aanvraag verder te kunnen beoordelen:

Onduidelijkheden
Kunt u per infectie of -groep aangeven hoe vaak deze voorkomt en wat de impact van de infectie is op de dieren? Kunt u ook aangeven of er al vaccins aanwezig zijn tegen deze infectie en waarom het nodig is nieuwe vaccins te ontwikkelen?

Bij uw strategie mist een onderbouwing van de keuzemomenten. Het is niet helder wanneer welke keuze gemaakt wordt. Bijvoorbeeld, wat is al bekend van de vaccins die u in vivo wilt gaan testen, voordat u deze vaccins in een dierproef gaat testen. Wanneer besluit u een nieuw diermodel op te zetten of een bestaand diermodel te verbeteren?
Wanneer besluit u niet verder te gaan met de ontwikkeling van een bepaald vaccin? Welke strategie zult u volgen voor optimalisatie van reeds bestaande vaccins en welke voor nieuw te ontwikkelen vaccins?

Kunt u voor alle Bijlage Dierproeven aangeven om welke redenen de huisvesting van dieren zonder substraat zal zijn, welke afweging wordt hierbij

gemaakt?

Hoeveel dieren ondergaan in Bijlage Dierproeven 3.4.4.1 en 3.4.4.2 matig ongerief vanwege herhaling van handelingen?

Kunt u voor Bijlage Dierproeven 3.4.4.3 aangeven hoeveel dieren welk ongerief zullen ondergaan? Kunt u aangeven welke infectiemodellen zullen leiden tot ernstig ongerief? U noemt enkele voorbeelden, maar kunt u dit uitbreiden?

Kunt u voor Bijlagen 3.4.4.3 met wetenschappelijke argumentatie aangeven waarom mannelijke dieren niet gebruikt kunnen worden in het experiment? Ongerief als gevolg van individuele huisvesting is niet voldoende argumentatie. Als onvoldoende is onderbouwd waarom het gebruik van één geslacht nodig is, kan de CCD een voorwaarde opleggen dat beide geslachten in gelijke mate gebruikt moeten worden.

Kunt u de humane eindpunten in alle Bijlage Dierproeven beter beschrijven, inclusief ziektespecifieke humane eindpunten?

Hoeveel vaccins/ infecties verwacht u in totaal te onderzoeken?

Kunt u dit verwerken in nieuwe Bijlagen Dierproeven en een nieuw Projectvoorstel?

Leges
De leges die u verschuldigd bent zijn nog niet door ons ontvangen of de betaling is nog niet verwerkt. Uw aanvraag is niet compleet als de leges niet zijn ontvangen.
Zonder deze aanvullende informatie kan de beslissing nadelig voor u uitvallen omdat de gegevens onvolledig of onduidelijk zijn.

Opsturen binnen veertien dagen
Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze brief op. U kunt dit aanleveren via NetFTP. Stuurt u het per post op, gebruik dan het formulier dat u bij deze brief krijgt.

Wanneer een beslissing
De behandeling van uw aanvraag wordt opgeschort tot het moment dat wij de aanvullende informatie hebben ontvangen. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn ontvangen. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Datum:
10 mei 2017
Aanvraagnummer:
AVD2210020171629

Met vriendelijke groet,

Centrale Commissie Dierproeven

10.

Geachte CCD,

Bijgevoegd zijn de antwoorden op gestelde vragen vanuit CCD n.a.v. brief 10 mei 2017.
Uw referentie is: Aanvraagnummer AVD2210020171629 m.b.t Project: Research of new ruminant vaccines.
In groen zijn de antwoorden weergegeven. Naar aanleiding van deze vragen zijn ook de documenten Project proposal, Bijlagen beschrijving dierproeven en de NTS aangepast. Ook hierin zijn de aanpassingen met groene tekst weergegeven.

We hopen u hiermee voldoende geïnformeerd te hebben. Indien er nog vragen zijn, dan vernemen wij deze graag.

Met vriendelijke groeten,

[redacted]

[redacted]

Op 2 mei 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Research of new ruminant vaccines" met aanvraagnummer AVD2210020171629. In uw aanvraag zitten voor ons nog enkele onduidelijkheden. In deze brief leest u wat wij nog nodig hebben en wanneer u een beslissing kunt verwachten.

Vraag: Onduidelijkheden
Kunt u per infectie of -groep aangeven hoe vaak deze voorkomt en wat de impact van de infectie is op de dieren? Kunt u ook aangeven of er al vaccins aanwezig zijn tegen deze infectie en waarom het nodig is nieuwe vaccins te ontwikkelen?

Antwoord:
Dit vaccine onderzoek project richt zich enkel op ziektes, die een beduidende impact op de herkauwer (rund, schaap, geit) industrie hebben (dierenwelzijn, productieverlies of behandelingskosten) en / of impact op humane gezondheid (zoönoses en reductie in het gebruik van antibiotica). Over het algemeen zal er alleen gewerkt worden aan ziektes met hoge incidentie in de (Europesche) runderpopulatie of aan exotische ziektes, die een bedreiging voor de Europesche runderpopulatie vormen.
Onder het kopje “Diseases within the Ruminant Vaccine Research Project” (3.1. Background) is hierover uitleg toegevoegd, tevens is voor de specifieke ziektes, indien van toepassing, informatie over incidentie, impact en / of beschikbaarheid en noodzaak voor vaccins toegevoegd.

Vraag:
Bij uw strategie mist een onderbouwing van de keuzemomenten. Het is niet helder wanneer welke keuze gemaakt wordt. Bijvoorbeeld, wat is al bekend van de vaccins die u in vivo wilt gaan testen, voordat u deze vaccins in een dierproef gaat testen. Wanneer besluit u een nieuw diermodel op te zetten of een bestaand diermodel te verbeteren? Wanneer besluit u niet verder te gaan met de ontwikkeling van een bepaald vaccin? Welke strategie zult u volgen voor optimalisatie van reeds bestaande vaccins en welke voor nieuw te ontwikkelen vaccins?

Antwoord:
Zoals reeds in de project proposal bij 3.4.3. aangegeven, zullen enkel projecten met een aannemelijke marktverwachting en kans op success opgestart worden.
Ter verduidelijking wordt de betreffende zin in 3.4.1. ook in toegevoegd: *“At the company,*

all R&D projects are subject to regular review by the R&D Governance Body. Only research projects with reasonable market expectation and probability of success will get approval to start the research phase.
At the start of a research project, the project team agrees on the types of studies to be performed and the requirements for the studies".
Verder is onder 3.4.3. informatie toegevoegd over verschillende keuzemogelijkheden omtrend de verschillende type studies (zie document).

Vraag:
Kunt u voor alle Bijlage Dierproeven aangeven om welke redenen de huisvesting van dieren zonder substraat zal zijn, welke afweging wordt hierbij gemaakt?

Antwoord:
Bij [redacted] infectiemodellen is het noodzakelijk, de faeces schoon te verzamelen, om deze visueel goed te kunnen beoordelen en bij voorbeeld de droge stof en / of de totale hoeveelheid faeces te kunnen bepalen. In dit type studies kan er darom geen substraat aangeboden worden. Tekst in Bijlage Beschrijving Dierproeven is bij 3.4.4.1 en 3.4.4.2. als volgt aangepast:
'In a few cases [redacted]), it is necessary to use no bedding because of scientific reasons'.
Dit is niet van toepassing voor Bijlage Beschrijving Dierproeven 3.4.4.3.

Vraag:
Hoeveel dieren ondergaan in Bijlage Dierproeven 3.4.4.1 en 3.4.4.3 matig ongerief vanwege herhaling van handelingen?

Antwoord:
Bij de beoordeling van het ongerief vanwege herhaling van handelingen werd voor de meeste procedures in eerste instantie een te strenge classificatie toegepast: Het herhaaldelijk nemen van [redacted] of meer dan [redacted] keer toepassen van de volgende procedures: meten van de lichaamstemperatuur, gewicht, nemen van urine, faeces, colostrum/melk of het bemonsteren van mucosale oppervlaktes [redacted], [redacted] werd als matig ongerief beoordeeld. Aangezien het niet-invasive handelingen betreft, is het te rechtvaardigen, het ongerief ter gevolge van herhaaldelijke toepassing van deze procedures als mild te beoordelen.
Ongerief ten gevolge van herhaaldelijke [redacted] punctie [redacted] punctie van [redacted] of [redacted] is echter wel als matig te beschouwen.
In de betreffende tabel in bijlage 3.4.4.1. waarin aangegeven wordt, hoeveel dieren welke ongerief zullen ondervinden zal worden aangegeven, hoeveel dieren (%) matige ongerief ter gevolge van de herhaalde procedures ondervinden. Boven de tabellen voor infectie modellen waar deze procedures niet toegepast zullen worden, wordt de *zin "due to repeated sampling, the cumulative discomfort score for the sampling is moderate"* verwijderd.
In de betreffende tabel in bijlage 3.4.4.2. waarin aangegeven wordt, hoeveel dieren welke ongerief zullen ondervinden zal worden aangegeven, hoeveel dieren (%) matige ongerief ter gevolge van de herhaalde procedures ondervinden. Bij de tabellen voor infectie modellen waar deze procedures niet toegepast zullen worden, wordt de *voetnoot "[2]: Although discomfort of disease is mild, overall study discomfort will be moderate as a result of repeated sampling"* verwijderd.
Onder Punt I is de tekst als volgt aangepast:
Measurement of body temperatures and body weight, sampling urine, feaces, colostrum/milk, sampling on different (mucosal) surfaces ([redacted], [redacted] as well as [redacted] and pregnancy check are part of normal farm practice/veterinary care and will induce only mild discomfort. If the following proceudres [redacted]) are repeated the discomfort is considered to be

moderate.
De verdeling van dieren over de verschillende ongeriefscores in de NTS zal ook worden aangepast.

Vraag:
Kunt u voor Bijlage Dierproeven 3.4.4.3 aangeven hoeveel dieren welk ongerief zullen ondergaan? Kunt u aangeven welke infectiemodellen zullen leiden tot ernstig ongerief? U noemt enkele voorbeelden, maar kunt u dit uitbreiden?

Antwoord:
Naar aanleiding van uw vraag realiseren we ons, dat de tekst mogelijk misleidend is. Er zijn infectie modellen die tot ernstig ongerief leiden ([redacted]), echter verwachten we gedurende de komende 5 jaar geen van deze modellen toe te passen. We verwachten dat niet meer dan [redacted] van de dieren matig ongerief zal ondervinden, ten gevolge van de handelingen of de infectie.
De tekst is als volgt aangepast:
'Discomfort will be mild (≥75%) to moderate (≤25%) depending on the number of sampling points, type of biotechnical procedure and whether the challenge infection causes disease in the model animal'.

Vraag:
Kunt u voor Bijlagen 3.4.4.3 met wetenschappelijke argumentatie aangeven waarom mannelijke dieren niet gebruikt kunnen worden in het experiment? Ongerief als gevolg van individuele huisvesting is niet voldoende argumentatie. Als onvoldoende is onderbouwd waarom het gebruik van één geslacht nodig is, kan de CCD een voorwaarde opleggen dat beide geslachten in gelijke mate gebruikt moeten worden.

Antwoord:
Bij de in bijlage 3.4.4.3 beschreven experimenten is standaardisatie van uitgesproken belang. Bij gebruik van dieren van beide geslachten wordt een additionele variable in de proefopzet toegevoegd, wat onvermijdelijk tot een grotere variabiliteit in de resultaten zal leiden. Om de zelfde mate aan betrouwbaarheid van de resultaten te waarborgen zou het aantal dieren dat per experiment nodig is verhoogd moeten worden. Gebruik van alleen vrouwelijke dieren leidt tot het totaal laagst mogelijk aantal dieren.

Vraag:
Kunt u de humane eindpunten in alle Bijlage Dierproeven beter beschrijven, inclusief ziektespecifieke humane eindpunten?

Antwoord:
Onderstaande informatie is toegevoegd in alle bijlagen:
General humane endpoints are applicable to all animals, irrespectively of the type of experiment.
General Humane Endpoints:

- *The animal experiences more than minor additional discomfort as a consequence of conditions resulting in long term or non-reversible inability to eat and or drink autonomously, fast or long lasting loss of weight, diseases or conditions that cause severe pain, suffering or discomfort such as bone fractions, force unnatural positioning and / or movements, open wounds or absesses.*
- *Scientific endpoints: The target of the study reached / all planned samplings have been performed*
- *(Reliable and useful) results cannot be reached for reasons unrelated to the study*

Onderstaande informatie is toegevoegd in alle bijlagen betreffende infectiestudies:
Specific humane endpoints after infection with bovine pathogens

- In general, disease specific clinical signs (for example [redacted] or [redacted] signs) do not normally lead to humane endpoints, but they may affect the general

health of the animal (i.e. high fever, dullness, anorexia) leading to humane endpoints if
    - severe general clinical signs last for at least 2 days or the body temperature drops rapidly
    - the animal is unable to stand up and eat/dring actively for more than 1 day
- In the reproductive infection models, calving or abortion are humane endpoints.

Vraag
Hoeveel vaccins/ infecties verwacht u in totaal te onderzoeken?

Antwoord
We verwachten gedurende de komende 5 jaar met [redacted] pathogenen development studies te verrichten (in vele gevallen zal een vaccin antigenen van meerdere pathogenen bevatten).

Zin toegevoegd in project plan onder kop 3.4. *"It is expected that [redacted] pathogens will be investigated within this project"*

Centrale Commissie Dierproeven

11.

Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	22100
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	Provide the title of the project.	Research of new ruminant vaccines

2 Categories

2.1 Please tick each of the following boxes that applies to your project.

- ☐ Basic research
- X Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or
- ☐ Research aimed at preserving the species subjected to procedures
- ☐ Higher education or training
- ☐ Forensic enquiries
- ☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

Rationale

Ruminants represent around 32% of the global animal health market, with vaccines being a key segment in the ruminant health market. This market is extremely diverse: the most important species of farmed ruminants are cattle, sheep and goat, but in certain regions, also buffalo, deer, and camels are raised in

an agricultural setting. The husbandry and management systems vary between species and also on whether the animals are kept for meat, milk or wool production. By consequence, a broad and diverse portfolio of ruminant vaccines is required to fulfil the specific needs for the different markets.
One aspect is however constant across the whole ruminant health market: the trend of increasing animal productivity and growing farm/herd size, which is worsening the impact of infectious diseases. Vaccination is widely applied to control infectious diseases, leading to a reduction in the amount of antibiotics used and better wellbeing for the animals.
The company is constantly striving to strengthen its portfolio of ruminant vaccines by improving existing vaccines and developing new ones. New fields of vaccine research may include microorganisms that are by themselves harmless for ruminants, but animals are vaccinated to reduce the risk of infection for humans for example in case of [redacted] diseases or other zoonoses. Vaccination for other reasons such as the [redacted] or other targets that are not infectious agents are out of scope for this application.

Each pathogen has its own specific mechanism of pathogenesis and protection through vaccination requires specific immune responses, either humoral, through cellular immunity, or a combination thereof. For this reason, new vaccines are to a large extent "tailor-made". Where possible, knowledge acquired with other pathogens / vaccines is used to design candidate vaccines in order to increase the likelihood of success and thereby minimize the numbers of animals needed during the whole development process of a new vaccine. Vaccines can be either live attenuated pathogens, (components of) inactivated pathogenes, [redacted]
Most inactivated vaccines are formulated together with an adjuvant and primarily induce a humoral response, while live (vector) vaccines typically induce both a humoral and cellular response. The attenuation of live vaccines can be accomplished by classical means (e.g. in vitro passaging or chemical mutagenesis) or by recombinant methods (GMO vaccines).

In case of diseases that affect very young animals, the mother is vaccinated in order to protect its offspring by the transfer of specific antibodies via the colostrum and milk or specific antibody preparations are added to the milk.

The two key requirements for any vaccine are (i) safety and (ii) efficacy. As vaccination is a medical treatment administered to healthy individuals, it is important that the vaccine does not cause undesired (negative) effects other than some transient minor discomfort. With regard to efficacy, the benefit in providing protection from disease and/or infection has to be demonstrated to justify the use. According to the applicable regulations in Europe and the majority of other countries, the safety and efficacy of a vaccine candidate has to be demonstrated in animal experiments. In addition, some quality control tests also require animal testing.

Vaccine research and development

Within a vaccine project two phases can be distinguished: the research phase and the development phase.
The research phase comprises the search for new pathogens, new adjuvants, new vaccine formulations and new [redacted] methods as well as expansion of the knowledge on known pathogens and technologies. For example, mechanisms of pathogenicity and natural and specifically induced immune responses or strategies of the pathogens to [redacted] will be investigated, protective antigens might be identified or methods of attenuation of strains to create live vaccines might be investigated. Candidate vaccines are then tested in pilot studies to determine their efficacy and safety. Based on the outcome of these studies, candidate vaccines with a fixed formulation are selected to enter the development phase.
In addition, (antigenic components of) the pathogens will be used to immunize laboratory animals to generate antibodies that can be used to set up immunological assays that are needed during the development phase
The current project proposal covers studies that are done during the research phase. The development phase is covered by a separate project proposal.
In general, the design of the development studies, including the procedures that have to be applied to demonstrate safety and efficacy of the vaccine are laid down in Guidelines and Regulations. The design and the procedures applied in the research studies are basically the same as for the development studies:

- Infection studies with new pathogens are performed to set up target animal infection models that can be used in efficacy studies in the development phase and to expand the knowledge on these

pathogens with regard to pathogenesis, immune responses, etc.
- Safety and efficacy research studies are performed in order to select vaccine candidates that are likely to successfully pass the development studies
- The objective of studies on *in vivo* potency testing during the research phase is to set up a test protocol for an in vivo potency test that can be applied during the development phase.

During the research phase, less knowledge and experience is available on the different pathogens / vaccine candidates / adjuvants. Therefore, higher discomfort scores may be reached in a larger proportion of animals.

A distinct type of studies is the immunization of laboratory animals to generate specific antibodies that can be used for immunological assays that are needed the indentification, detection and/or quantification of the antigen/pathogen. The commonly applied design and procedures are followed in these studies.

Below, some back-ground information is given about infectious diseases in ruminants that are in scope for this project.

Diseases within the ruminant vaccine research project

This vaccine research project targets diseases that have a major impact on the ruminant industry (animal welfare, production losses, treatment costs) and / or Human Health concerns (zoonotic disease and reduction in the amount of antibiotics used). Typically, only diseases with a [redacted] in the (European) cattle population or [redacted] for which the [redacted] are in scope. Diseases with a [redacted] but causing severe disease in animals and / or humans may also be considered.

Currently, the main target species for ruminant vaccines are cattle, yet several cattle pathogens also affect sheep and goat. In these cases, testing in small ruminants might also be necessary, especially in case of zoonoses or animal pathogens that are subject of control programs. Moreover, small ruminants might serve as model for infection studies.

The following infectious diseases are targeted within the ruminant vaccine development projects in the company.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

Current products, R&D projects and historical progress

[redacted]

[redacted]

3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

The goal of the ruminant vaccine research project is to update the current [redacted] vaccine portfolio in response to unmet needs in the field of ruminant livestock industry. More specifically, the aim is to identify new pathogens, expand the knowledge of known pathogens to be able to develop new (combination) vaccines and test candidate vaccines against these pathogens. The outcome of a successful research phase is the discovery of a new pathogen and/or a prototype vaccine that has shown to be safe and efficacious in the target animals (proof of concept) and will therefore later on successfully pass the development phase. In addition, (antigenic components of) the pathogens will be used to immunize laboratory animals to generate antibodies that can be used to set up immunological assays for the detection and/or quantification of the antigen/pathogen and the immune response against the antigen/pathogen (e.g. ELISAs, serotyping tests, immunohistochemistry, humoral and cellular immune responses).

3.3 Relevance

What is the scientific and/or social relevance of the objectives described above?

Vaccines are the most effective method for prevention or eradication of diseases. Further improvement and extension of the available vaccine range will bring safer, more efficacious vaccines, including vaccines against emerging diseases. Also, combinations of diseases can be encountered more effectively, with fewer vaccination moments (injections), when vaccines are developed that can be used at the same time or mixed with other vaccines, or even in ready to use combination products.
The prospects are that the new vaccines will further reduce animal suffering and the use of antibiotics, and will lead to reduced losses in meat and milk production and thereby to a more sustainable use of natural resources.
Acquired insight in pathogensis and immune responses of infections will help to identify new vaccination strategies for those pathogens for which no efficacious vaccine exists at this moment.

3.4 Research strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

At the company, all R&D projects are subject to regular review by the R&D Governance Body. Only research projects with reasonable market expectation and probability of success will get approval to start the research phase.
At the start of a research project, the project team agrees on the types of studies to be performed and the requirements for the studies.
Infection studies in the target animal (ruminants) have to be undertaken to show that a (newly isolated) infectious agent is pathogenic and fulfils Koch's postulates and to better understand the mechanisms of pathogenicity and immune response after infection. These infection studies can also form the basis for a target animal (ruminants) infection model that will be used to test the efficacy of vaccine candidates (vaccination-challenge studies). Such an infection model is needed to be able to show that a vaccine is capable to prevent or significantly reduce infection and/or clinical signs. For vaccines against some pathogens, the infection model that has to be used and the specific efficacy criteria that have to be fulfilled are prescribed in a monograph of the Ph.Eur.
Due to the complexity of vaccination-challenge studies in ruminants, the number of vaccine candidates

tested in vaccination-challenge studies is reduced as much as possible. [redacted]
When doing (initial) vaccination-challenge studies, it is attempted to find an [redacted] so that in further studies efficacy can be evaluated on the basis of [redacted].
Safety of vaccine candidates has also to be evaluated in the target animals (ruminants) to show that systemic and local (injection site) reactions after vaccination, if any, are acceptable. For each new vaccine, a risk-benefit analysis has to be made and the aim is to induce as little as possible discomfort by vaccination.
In the research phase, safety and [redacted] efficacy parameters are measured simultaneously in combined orientating efficacy and safety studies.
It is expected that [redacted] pathogens will be investigated within this project.

3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

The research phase for new vaccine consist of one or more of the following types of animal experiments (described in detail in appendices 1 through 3).

Ad 1) Infection studies
An infection model for a pathogen is developed based on the scientific literature or a Ph.Eur monograph (if available) and the experience with other pathogens within the company. In a model, it will be attempted to reproduce the clinical signs that are associated with a certain disease or syndrome. For a potentially new pathogen this will reveal if it is indeed able to induce disease (Koch's postulates). For known pathogens the model has to allow assessment of the efficacy of vaccine candidates under controlled laboratory conditions as described in Ph.Eur 5.2.7 (Evaluation of efficacy of veterinary vaccines and immunosera) or vaccine specific monographs, EU Directive 2009/9/EC amending Directive 2001/82/EC (Community code relating to veterinary medicinal products) and national guidelines and regulations outside the EU. New infection models are defined as models for newly discovered (potential) pathogens or published models that have not been used within [redacted] before. Improvement or refinement of existing models will be undertaken in case not all disease characteristics that are relevant for the field situation are presented in the model, or if the model shows a high variability in the level of infection/pathology within a group of infected animals and which would therefore necessitate the inclusion of large groups of animals in infection-challenge experiments in order to be able to show statistically significant differences. In case of multifactorial diseases, it might be necessary to [redacted] effect of [redacted] factors in order [redacted] pathogen.For this purpose, [redacted] drugs might be applied or the animals are co-infected with [redacted] pathogens.
Testing of new serotypes/pathotypes of a pathogen is also considered improvement rather than development of a new model, as the route of application etc. will be based on experience present within the company. Furthermore, refinement will also include the testing of modifications to a model with the intention to increase animal welfare (e.g. a less invasive application method).

Studies to investigate the pathogenicity and / or to develop or improve an infection model will have the following set-up:
- Administration of a (potential) pathogen, if required in combination with [redacted]
- Observation of clinical signs post infection/sampling (e.g. for shedding of the pathogen, immune responses against the pathogen)
- Necropsy to investigate (histo)pathological changes

The degree of discomfort will depend on the nature of the pathogen involved as the infection model is supposed to mimic the natural disease as much as possible.

Ad 2) Vaccination studies
Once an infection model has been established, the efficacy of candidate vaccines against a pathogen can be evaluated. When looking into the options for vaccination against a newly discovered pathogen or a pathogen for which no vaccine is available, the vaccine candidate(s) to be tested are based on the scientific literature and the knowledge within the company on pathological processes, immune mechanisms and vaccines against related pathogens to have the highest chance of success and thereby minimize the number of animals needed. This will determine whether a live or inactivated vaccine

approach will be taken. In some instances, there will be collaboration with outside partners (e.g. universities) that have specific knowledge on a (new) pathogen and that might even already have prepared and tested vaccine candidates. In addition, research on new (combination) vaccines for pathogens that are already being controlled by vaccination will also be guided by the experience gained under field conditions with the marketed product(s). An inactivated vaccine can be a whole killed microorganism or virus, or an immunogenic part of the pathogen (subunit vaccine). Also [redacted] vaccines or vaccines [redacted] fall into this category. An inactivated vaccine will be formulated with an adjuvant that is expected to be safe in the target animals (ruminants) and if applicable the laboratory animal used for the potency testing in combination with the chosen antigen(s) and will be quality control tested (e.g. for sterility) before the start of an animal experiment to reduce the chances of unwanted vaccination reactions. A live vaccine is an attenuated form of the pathogen that has been prepared by "classical methods", such as cell culture passage or chemical mutagenesis, or by targeted gene modifications with the help of recombinant-DNA techniques. Another form of live vaccines, are so called vector vaccines that consist of either or non-pathogenic microorganisma or an attenuated pathogen that also contains antigen(s) of other pathogens. A live vaccine candidate will be characterized and tested for purity before the start of studies in animals. In vaccination-challenge studies using the infection model, it will be evaluated whether the vaccine candidate can provide the required protection against the pathogen in terms of reduction of infection, clinical signs and (histo)pathology. Only if a vaccine candidate gives promising results (i.e. (statistically significantly) reduces one or more aspects of a disease) it is considered for the development phase. By studying the immune response after vaccination, it will be attempted to find a correlation between the height of the immune response (e.g. as measured in in vitro virus-neutralization) and protection in the target animal (ruminant). In those instances where such a correlation can be established, candidate vaccines can be tested on the basis of that response instead of by challenge infection. However, although some vaccines are able to protect against the disease in question, the immune response measured (if any) is not always indicative of the level of protection, especially in case protective antigen(s) are unknown. If no correlate of protection is available, vaccine efficacy can only be evaluated in vaccination-challenge studies.
In order to make a proper risk-benefit analysis for a new product, all vaccines have to be tested in safety studies in the target animal (Ph.Eur 5.2.6 (Evaluation of safety of veterinary vaccines and immunosera), EU Directive 2009/9/EC amending Directive 2001/82/EC (Community code relating to veterinary medicinal products), VICH guidelines and national guidelines and regulations). Inactivated and subunit vaccines usually contain an adjuvant that enhances the immune response to the antigen(s) in the vaccine. Unfortunately, although the adjuvant preparations themselves can be considered safe, the combination of antigen and adjuvant sometimes results in unwanted systemic and/or local reactions after vaccination. Therefore, for each new inactivated or subunit vaccine the effect on the animals' general health, determined by observing clinical signs (e.g. general demeanour, body temperature, appetite etc.) and injection site reactions has to be determined. For an attenuated live vaccine, it has to be shown that it is unable to induce disease. Therefore, live vaccine candidates will be evaluated for their lack of virulence in the infection model. Testing specific gene-deleted mutants in the infection model will also provide knowledge on which antigens are required for pathology and/or survival within the host. These antigens can then be considered for an inactivated vaccine approach.

As evaluation of the safety and efficacy of vaccination will be combined in the research phase, studies will be performed according to the following basic set-up (infection will not be performed in case an immunological marker for protection can be applied):
- Administration of the candidate vaccine
- Observation of clinical signs post vaccination
- Monitoring responses (e.g. blood sampling)/persistence (e.g. shedding) in case of a live vaccine
- Infection with a pathogen (field isolate)
- Observation of clinical signs post infection/sampling (e.g. for shedding of the pathogen)
- Necropsy to investigate (histo)pathological changes

The degree of discomfort encountered directly as a result of vaccination and sampling is small and such procedures are routinely used in normal veterinary care. The one area in which a moderate to severe degree of suffering may occur is after the onset of clinical disease following infection.

Ad 3) Assay development and preparation of biomaterials
To set-up assays that can help to detect a pathogen (e.g. by immunohistochemistry),to discriminate between different strains (e.g. serotyping) or develop immunological assays to quantify whole pathogens or specific antigens (antigenic mass and potency tests) it may be necessary to use laboratory animals for the preparation of sera or monoclonal antibodies if the required reagents are not available. For each new

vaccine, batch tests for the quantification and identification of the active ingredients are required under EU Directive 2009/9/EC amending Directive 2001/82/EC (Community code relating to veterinary medicinal products) and Ph.Eur 0062 (Vaccines for veterinary use) to verify the consistency of the manufacturing process and the final product. Preferably, in vitro tests are used for batch testing, but in case an in vitro batch potency test is not possible for a new vaccine, a serological assay in laboratory animals will have to be set up. In addition, for some vaccines for which a Ph.Eur monograph exists, a mandatory batch potency test in laboratory animals is described.
In a few of cases, determination of the protection against challenge infection is mandatory or necessary for scientific or technical reasons.

Immunization experiments of laboratory animals will generally be as follows:
- Administration of antigen/pathogen
- Collection of blood

In case of potency tests that involve challenge infection, the following additional treatments are employed
- Administration of challenge inoculum
- Clinical observation

The degree of discomfort encountered directly as a result of vaccination and sampling is small and such procedures are routinely used in normal veterinary care. In the studies without challenge, discomfort will be mild or moderate depending on the number of injection/sampling moments.
Moderate to severe degree of suffering may only occur in studies with challenge infection.

3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points.

At the company, all R&D projects are subject to regular review by the R&D Governance Body. Only research projects with a reasonable market expectation and probability of success will get approval to start the research phase.
At the start of a research project, the project team agrees on the types of studies to be performed and the requirements for the studies.

The Type of experiments and go / no-go decision points in a research vaccine project are the following:

1. Infection studies.

The appearance of new strains of a particular pathogen is often difficult to observe, and can rely on anecdotal reports from veterinarians and animal owners. Field isolates might therefore have to be tested both *in vitro* and *in vivo* to determine if significant changes in pathogenicity have occurred. Studies are performed to investigate the pathogenicity of an agent and the immune response induced by the agent. If a potential pathogen fails to fulfil Koch's postulates, the vaccine research project will be stopped.
Infection studies with field isolates are also performed to develop or improve an infection model. An infection model is required for vaccine development (see 2. Vaccintion challenge studies). Reasons why an existing model needs to be improved could be that the model is too artificial and does not mimic the natural disease or because of a too large animal – to - animal variation in the results. In case of multifactorial diseases, it might be necessary to [redacted] effect of [redacted] factors in order to [redacted] pathogen. As it is important to have the smallest possible variation in the level of infection/clinical signs between animals to be able to work with the lowest number of animals possible in the challenge experiments performed during the development phase, improvement/refinement of the challenge model (e.g. change in the route of inoculation) may need to be undertaken. Also, when a new challenge inoculum is prepared, suitability for use in challenge studies has to be evaluated in the model. On the other hand infection studies are required to determine whether a strain is suffiently attenuated to serve as vaccine strain. On the basis of the study results, it will be determined if a live vaccine candidate has an acceptable risk-benefit profile. Some fine-tuning of the composition (e.g. changes in dose or application route) may be necessary before the optimal vaccine has been reached (proof of concept). In some cases it may not be possible to obtain proof of concept with the available candidates and knowledge of the pathogen, which means that the research project will be stopped.

2. Vaccination challenge studies

Vaccination challenge studies can only be performed once an infection model (see point 1) is available.
Orienting vaccination challenge studies are performed to get a first impression about the safety and efficacy of the vaccine candidates. It may take several rounds of experiments to test a number of vaccine candidates in order to find a candidate vaccine that has proven to be able to fulfil the required efficacy and safety criteria, the vaccine candidate can move to the development phase. If, with the knowledge available, it is impossible to produce a candidate vaccine that fulfils the criteria, further development is

stopped.

3. Studies in laboratory animals for assay development and preparation of biomaterials.

These studies can be initiated in parallel with studies mentioned under 1. And 2., but they will only be undertaken if the necessary immunological reagents are not (commercially) available. A potency test in laboratory animals (according to Ph.Eur monographs) will only be developed in case a no suitable *in vitro* test is available.

All above mentioned studies are eventually done to obtain regulatory approval for ruminant vaccines. During the regular (1-2 times per year) project reviews by the R&D Governance Body the outcome of the studies is assessed against the requirements and pre-set milestones as well as go/no-go decision points are evaluated.

3.4.4 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

Serial number	Type of animal procedure
1	Infection studies in ruminants
2	Vaccination challenge studies in ruminants
3	Assay development and preparation of biomaterials
4	
5	
6	
7	
8	
9	
10	

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	22100
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 1 — Type of animal procedure: Research: Infection studies in ruminants

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

Infection studies will be performed for one of the three different reasons:

1) To determine the pathogenicity of new pathogens (to prove Koch's postulates), variants of known pathogens, should they appear or the role of specific genes in the pathogenicity and interaction with the host's immune system.
2) To develop an infection model that will be used in vaccination-infection studies to assess the efficacy of vaccine candidates (see Addendum 2).
3) To assess the safety profile of a live attenuated vaccine candidate obtained by "classical" means (e.g. in vitro passage or chemical mutagenesis) or gene-modification (including vector vaccins).

In general, the application of a pathogen will be done via the natural route of infection, but if the natural route does not induce all presentations of a disease under laboratory conditions, it might be necessary to use another route (e.g. parenteral injection to induce a systemic infection).
Application of a potential vaccine candidate will typically be done by the route intended as application route of the future product, but in specific cases it might be necessary to follow the route that gives highest risk of adverse events in order to make a meaningful assessment of the safety profile.
In case a pathogen or vaccine candidate might cause transplacental infection, pregnant animals at one or more specified stage of pregnancy have to be used in the infection studies. The animals are then observed until the end of pregnancy to determine the outcome of the pregnancy and the health status of the offspring. Samples might have to be taken from the offspring to determine the presence of the pathogen / vaccine candidate or specific pre-colostral antibodies. Alternatively, [redacted]

After inoculation of the vaccine candidate or pathogen, one or more of the following parameters will be

evaluated:

- Clinical signs (e.g. changes in general health and or disease specific symptoms and local reactions)
- Body temperature (rectal temperature)
- Body weight
- Virus, bacterial or parasites shedding (swabbing of mucosal surfaces, sampling of faeces, urine, milk), viral/bacterial/parasitic load in [redacted] or other tissues ([redacted])
- Viraemia, bacteraemia or parasitemia or haematological parameters (blood/ [redacted] sampling)
- Post mortem examination (macroscopical and microscopical)

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Four or more of the following procedures will be undertaken depending on the characteristics of the vaccine/ pathogen involved; the types of pathogens are described under B (in italics the frequency of the procedures):

1. Daily observation / scoring clinical signs including measurement of rectal temperature ([redacted] *for up to* [redacted] *days)*
2. Weighing [redacted]
3. Blood sampling [redacted] to determine [redacted] and / or to determine the presence of the pathogen or vaccine candidate in the blood
4. [redacted] treatment by application of [redacted] drugs ([redacted] / [redacted]
5. Administration of pathogen or vaccine candidate [redacted]
6. Swabbing of (mucosal) surfaces [redacted] *)* to determine the excretion of the pathogen or vaccine candidate
7. [redacted] to determine the presence of the pathogen or vaccine candidate [redacted]
8. [redacted] to determine the presence of the pathogen or vaccine candidate in the [redacted]
9. Urine, fecal, colostrum / milk samples [redacted] to determine the presence of the pathogen or vaccine candidate
10. Punction of [redacted] *)* or installation of a [redacted]
11. Punction [redacted]
12. [redacted] biopsy [redacted]
13. Pregnancy check [redacted]
14. Euthanasia

The duration of all procedures listed above will only be minutes at most. Typically, the length of the observation period after infection is 14 to 28 days. If pregnant animals have to be followed until birth, the observation period will be longer, but in general, the total number of treatments does not increase.
To rule out that clinical signs are caused by an unintended co-infection, non-infected control animals may be included in a study. In addition, in models for neonatal disease in sheep and goats, ewes/goats may be required to give birth to and foster the lambs / kids but these will not be infected.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

In this type of initial studies, it is not obligatory to demonstrate statistically significant differences between treatment groups. However, these studies will enable an estimation of the variance between individual responses of the animals in a group at these particular observation points. Based on this information the minimum numbers of animals per group needed to demonstrate the efficacy of a vaccine during the development phase can be estimated. In this type of experiments, the group size is in general 5-8 animals, depending on the expected variation in the infection model. This group size is in line with the group size for infection studies as specified in most European Pharmacopoeia monographs on ruminant vaccines.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Studies will be performed in cattle, sheep and / or goats as appropriate. Animals of both sexes can be used for this type of animal experiment unless the study has to be performed in pregnant and / or lactating animals.

Purchase of animals: The animals will be purchased from commercial suppliers, obtained from affiliated farms or bred at [redacted] facilities.
If a certain microbial status is required, animals will be purchased from farms with the respective (certified) microbiological status and / or screened prior to inclusion in the study.
If the study design requires that animals are vaccinated and / or infected [redacted] or when only a [redacted], the young animals have to be [redacted] accordingly from the farms of birth to the testing facility.

Special requirements
If young animals with a specific antibody status are required, it is often necessary to warrant that [redacted]. As these [redacted] animals are more susceptible for intercurrent infections, they receive [redacted] treatment. With the exception of studies against [redacted] pathogens, [redacted] can be given to the animals from [redacted] onwards as an aid in the prevention of [redacted]

Age of animals:
Age of the animals for vaccine research varies from a few hours old to adult. The age of the animals to be used should be the age at which clinical disease is expected or the minimal age recommended for use of the vaccine.
If the infection has to be done in very young lambs or kids, it might be necessary to include the ewes / goats to foster the lambs / kids. In order to study transplacental spread of the pathogen or vaccine

candidate it may be necessary to include dams in one or more specific trimester of pregnancy. Samples might have to been taken from the offspring, for certain studies, euthanasia of the offspring might be required.

The tables below specify which models would be used for the different pathogens that might be included in infection studies during the research projects over the next 5 years. The lists are more extensive than the actual portfolio will be, but it is not possible at this moment to predict exactly which pathogens will be worked on.
The animal categories listed are the age groups considered to be most sensitive and therefore have to be used to perform the basic efficacy and safety studies.

Priorities within the R&D portfolio are based on market needs and the estimated likelihood of success of obtaining a vaccine candidate that fulfills the required product profile. Priorities can shift upon identification of new unmet needs in the field. For example, if a new [redacted] pathogen with substantial impact on the ruminant industry is discovered, this will be given priority over research into a second generation improved product for a [redacted] pathogen.

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 2 wk
New pathogen	Cattle <6 Months old	Severe (max 70%)[1]	Max 2 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 2 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 2 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 50%)	Max ± 2 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
New pathogen	Cattle <6 Months old	Moderate (max 100%)[2]	Max 2 wk

[1]Vaccines are given to [redacted] in order to [redacted]. This part of the study does only cause mild discomfort. The information given in the table above relates to the discomfort during challenge studies [redacted] will be determined.

[2]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 50%)	Max 2 wk
[redacted]	Adult cows	Moderate (max 50%)	Max 2 wk
[redacted]	Adult cows	Mild	Max 2 wk
[redacted]	Adult cows	Mild	Max 2 wk
[redacted]	Adult ewes	Mild	Max 2 wk
[redacted]	Adult ewes	Mild	Max 2 wk
New pathogen	Adult cows	Moderate (max 100%)[1]	Max 2 wk
New pathogen	Adult ewes	Moderate (max 100%)[1]	Max 2 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
New pathogen	Adult cows	Severe (max 100%)[1]	Max 1 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle <6 Months and adult cows	Moderate (max 50%)	Max 2 wk

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	sheep <6 Months	Severe (max 40%)	Max 1-2 d
	cattle<6 Months	Moderate (max 70%)	Max 2 wk
[redacted]	sheep <6 Months	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 70%)	Max 1-2 days
[redacted]	sheep <6 Months	Severe (max 50%)	Max 1 wk
[redacted]	cattle<6 Months	Moderate (max 70%)	Max 1 wk
[redacted]	sheep <6 Months	Mild	Max 2 wk
	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Severe (max 50%)	Max 1-2 d
[redacted]	sheep < 6 Months	Severe (max 50%)	Max 1-2 d
[redacted]	cattle<6 Months sheep < 6 Months	Mild	Max 2 wk
New pathogens and new types of models ([redacted])	cattle<6 Months sheep < 6 Months	Severe (max 100%)[2]	Max 1 w

[1] Efficacy of [redacted] vaccines in ruminants is tested by [redacted] only

[2]: Studies with potential new pathogens are given the maximum discomfort score until the severity of clinical signs has been established

Based on the experience over the last 5 years and the current R&D program and priorities, the total expected number of cattle, sheep and goat per age group and discomfort category is the following:

Species	Discomfort score*	Animals<6 months	Adult**
Cattle	Mild	[redacted]	[redacted]

	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]
Sheep	Mild	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]
Goat	Mild	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]

*: Discomfort due to disease

**: Including [redacted]

***: Due to repeated procedures the overall discomfort will be moderate in at most 50% of the animals [redacted]

In the infection studies, one or more groups are compared to an uninfected control group. The group size is dependent on the disease model. In general, the group size used will be [redacted] [redacted] but could be larger depending on the expected variation in the infection model. The expected numbers per category of vaccine are:

Per Model	Cattle <6Months	Adult cattle	Sheep <6Months	Adult sheep	Goat <6Months	Adult goat
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

C. Re-use

Will the animals be re-used?

☐ No, continue with question D.

X Yes > Explain why re-use is considered acceptable for this animal procedure.

Re-use might be considered for animals that experience only minor discomfort during the first study, for example uninfected control animals. This approach is considered acceptable especially in case of animals that were raised under special conditions (e.g. [redacted]) to achieve a certain [redacted] status

Are the previous or proposed animal procedures classified as 'severe'?

xNo

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research

strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:
In accordance with international regulations, animals of the target species must be used to demonstrate the safety and efficacy of a vaccine because there are no suitable alternatives or models for the induction of immunity in a whole organism or for the infection of living tissues as complex as those found in the whole animal in which the vaccines are intended to have efficacy. Therefore, during the research phase, infection models have to be developed in the target animals-and candidate vaccines have to be tested in the same models.

Reduction: All studies are performed with the lowest possible number of animals that are required to enable meaningful interpretation of the results. This will be achieved through an ongoing evaluation of the observations in each study. The number of animals per study will be substantiated in each study protocol. According to internal procedures, the study protocol will be reviewed by the Animal Welfare Body and a statistician.

Refinement:
Where possible it is pursued to refine the routes of administration of substances and sampling techniques to improve animal welfare/to reduce discomfort of administration, but without endangering the scientific outcome. Ruminants are the target species and there are no other less innervated/sentient species that could be a model for the ruminant diseases that are studied. See next paragraph for other refinement methods that are applied.
The classic method to prove protection of a new vaccine is efficacy in a vaccination-challenge test. However, if immunological correlates of protection (e.g. a serological response) can be used to prove efficacy this will be used rather than challenge infection. When an infection model has to be used, humane endpoints will be employed and staff will be fully trained to recognize animals that experience discomfort. Animals will be closely monitored and additional health checks are performed to ensure that no animal is left suffering.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

For monitoring of the clinical health status of animals, all study animals will be checked at least once a day by a certified person. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.
Furthermore efforts are made to optimally enrich the environment during containment.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

The vaccines in the companys R&D program are unique and proprietary to the company. To show that vaccines are compatible (combined or associated use), a number of safety and efficacy studies done with the individual products has to be repeated with the vaccines administered together according to international regulations and guidelines.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[] No

X Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

The animals are housed socially, but animals might have to be housed (temporarily) [redacted] in order to [redacted]

[redacted]. Some studies may require limited bedding during containment. In a few cases ([redacted] model), it is necessary to use no bedding because of scientific reasons.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

XYes > Will anaesthesia, analgesia or other pain relieving methods be used?

[] No > Justify why pain relieving methods will not be used.

Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Injections (for application of challenge material, injection with [redacted] drugs or agents) as well as sampling of blood are part of normal farm practice/veterinary care and will induce only mild discomfort. If the sampling is repeated (>5), the discomfort is considered to be moderate as a result of the stress when restrained and during handling of the animal.
All biotechnical procedures such as vaccination and blood sampling procedures have been described in Standard Operating Procedures (SOPs) (GLP accredited procedures).

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Measurement of body temperatures and body weight, sampling urine, feaces, colostrum/milk, sampling on different mucosae [redacted] as well as [redacted] and pregnancy check are part of normal farm practice/veterinary care and will induce only mild discomfort.
If the following proceudres [redacted] sampling or n[redacted] brushes, punction [redacted], punction [redacted] biopsy) are repeated the discomfort is considered to be moderate.
All biotechnical procedures such as inoculation and sampling procedures have been described in Standard Operating Procedures (SOPs) and only well trained personnel will be responsible for the execution (GLP accredited procedures).
[redacted] animals are more susceptible to disease and might therefore encounter discomfort related to intercurrent diseases. Transport of the animals to the testing facility might cause transient discomfort for the duration determination the protection against challenge infection of the transport, especially for animals that are transported [redacted].
The pathogenticiy of the pathogen / safety profile of the vaccine candidate used in the infection studies is not yet determined. Therefore, it is possible that adverse events occur.
Depending on the nature of the pathogen / vaccine candidate the discomfort of the infection can range from mild in the absence of any clinical signs (e.g. [redacted]) to moderate (e.g [redacted] and severe (e.g. [redacted]).

In case an infection model has to be developed for pathogens that do not cause clinical disease or only very mild disease, it might be necessary to [redacted]. These procedures and any

effects related to the [redacted] treatment are already taken into consideration for the discomfort levels and durations listed above in the respective tables.
The clinical health status of all animals is checked at least once a day by qualified personnel. Special attention is paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.
In consultation with the veterinarian and Study Director, if treatment does not interfere with the test results, it will be decided whether to apply adequate veterinary care including analgesia to alleviate treatment related pain (for example infection studies with [redacted] pathogens or [redacted] or pain not related to the treatment. In case of severe suffering, humane endpoints are applicable. General humane endpoints are described in an SOP (e.g. the condition of the animal prevents it from eating and drinking regularly, severe loss of body weight) and test specific humane endpoints are given in each study protocol if applicable.

Explain why these effects may emerge.

These procedures may be part of the experimental design. For vaccines intended for use in young animals, the study design may require that animals are vaccinated and / or infected at [redacted] or when only a[redacted]. In these cases, the young animals have to be [redacted] accordingly from the farm of birth to the testing facility.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

All biotechnical procedures will only be performed according to standard procedures described in SOPs (GLP accredited procedures).
The number of samplings is reduced to a minimum number required to for a valid evaluation of results.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

The severity of discomfort is depending on the nature of the pathogen (see 3.1 of the project proposal for specific clinical signs of the pathogens involved). However, the duration of severe discomfort will be limited due to the application of a humane endpoint if needed.
General humane endpoints are applicable to all animals, irrespectively of the type of experiment.
General Humane Endpoints:

- The animal experiences more than minor additional discomfort as a consequence of conditions resulting in long term or non-reversible inability to eat and or drink autonomously, fast or long lasting loss of weight, diseases or conditions that cause severe pain, suffering or discomfort such as bone fractions, force unnatural positioning and / or movements, open wounds or absesses.
- Scientific endpoints: The target of the study reached / all planned samplings have been performed
- (Reliable and useful) results cannot be reached for reasons unrelated to the study

Specific humane endpoints after infection with bovine pathogens

- In general, disease specific clinical signs ([redacted]) do not normally lead to humane endpoints, but they may affect the general health of the animal (i.e. high fever, dullness, anorexia) leading to humane endpoints if
  - severe general clinical signs last for at least 2 days or the body temperature drops rapidly
  - the animal is unable to stand up and eat/dring actively for more than 1 day
- In the [redacted] are humane endpoints.

These test-specific humane endpoints are described in the corresponding study protocol. Each study protocol is reviewed by the AWB before execution of the study.
In case it is difficult to reach a decision based on the pre-defined criteria for an endpoint the designated veterinarian is empowered to decide that a humane endpoint is applied/reached.

Indicate the likely incidence.

Considering the expected number of studies with the different pathogens, the expected number of animals included in the different treatment groups (i.e. infected vs control group) and the expected

severity, at most 30 % of the animals is expected to have severe discomfort that would require euthanasia.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

For the infection studies the type and severity of the clinical signs are depending on the type of disease. Similar to natural field infections they may cause mild to severe pain, distress, suffering or even impending death. See B for an overview of the different pathogens involved, the maximal discomfort caused by the disease and the maximum number of animals expected to reach the highest discomfort category.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Postmortem investigation can be part of the experimental design to evaluate (histo)pathological lesions at different organ systems and or to attempt re-isolation of the inoculum from tissues and organs.
In addition, animals with a pathogen cannot be returned to the farm of origin or transported to another farm to prevent the spread of disease. Therefore, all animals might have to be euthanized at the end of the study or when a humane endpoint is reached. Control animals that have not been infected may be reused or returned to the farm of origin or transported to another farm.
Moreover, return of the animals to commercial farms or slaughter for human consumption is often prohibited by the current legislation on use of antibiotics.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	22100
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: Research: Vaccination challenge studies in ruminants

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

During the research stage of a vaccine project, studies are carried out to estimate, whether the candidate vaccine is likely to successfully pass the safety and efficacy studies that are later on performed in the development phase.
The aim of this type of vaccination studies is to test if a vaccine candidate i) has acceptable safety characteristics and ii) is efficacious i.e. induces a measurable immune response and / or protects against one or more aspects of the disease caused by the pathogen(s) involved. To this end, animals are vaccinated according to the anticipated schedule and observed for local and systemic reaction after vaccination. The immune response after vaccination is determined. In the absence of a correlate of protection, the vaccinated animals or in case the vaccine is intended for the induction of passive protection, animals fed colostrum from vaccinated mothers will be infected with a challenge strain according to the previously established infection model (see [redacted] Unvaccinated ~~a~~animals will also be included in the experiment as control to determine the efficacy of the experimental vaccine. After vaccination and after challenge infection, one or more of the following parameters will be evaluated:

- Clinical signs (e.g. changes in general health and or disease specific symptoms and local reactions)
- Body temperature (rectal temperature)
- Body weight
- Virus, bacterial or parasites shedding (swabbing of mucosal surfaces, sampling of faeces, urine, milk), viral/bacterial/parasitic load in [redacted] or other tissues [redacted]
- Viraemia, bacteraemia or parasitemia or haematological parameters (blood/[redacted] sampling)
- Post mortem examination (macroscopical and microscopical)

On the basis of the study results, it will be determined if a vaccine candidate has an acceptable risk-benefit

profile that is in line with criteria that have been laid down in EU directives, the Pharmacopoeia Europaea (Ph.Eur) and guidelines and regulations of the European Medicines Agency and other international regulatory bodies when applicable. Some fine-tuning of the composition (e.g. changes in antigens/adjuvant included) may be necessary before the optimal vaccine has been reached (proof of concept). In some cases it may not be possible to obtain proof of concept with the available candidates and knowledge of the pathogen, which means that the research project will be stopped.
During these orientating vaccination studies, it will also be attempted to find a serological correlate with protection or another surrogate immunological marker that will enable the drawing of conclusions on the efficacy of a candidate vaccine without challenge in further studies.
In case of [redacted] diseases, it might be necessary to mimic the [redacted] effect of [redacted] factors in order to reach the required sensitivity of the animals to the challenge infection with [redacted] pathogen.
In case a vaccine is intended for protection against transplacental infection, pregnant animals are challenged at one or more specified stage of pregnancy. The animals are then observed until the end of pregnancy to determine the outcome of the pregnancy and the health status of the offspring. Samples might have to be taken from the offspring to determine the presence of the challenge strain or specific pre-colostral antibodies. [redacted]

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Four or more of the following procedures will be undertaken depending on the characteristics of the vaccine/ pathogen involved; the types of pathogens are described under B (in italics the frequency of the procedures):

1. Daily observation / scoring clinical signs including measurement of rectal temperature *(*[redacted]
2. Weighing [redacted]
3. Blood sampling [redacted] to determine [redacted] parameters and / or to determine the presence of the vaccine strain in the blood and / or to determine the presence of the challenge strain in the blood
4. Vaccine administration ([redacted]
5. Palpation of the injection site [redacted] *)*
6. [redacted] treatment by application of [redacted] drugs [redacted] and / or inoculation of [redacted]
7. Challenge administration [redacted]
8. Swabbing of (mucosal) surfaces [redacted] to determine the excretion of the vaccine and / or [redacted]) to determine the excretion of the challenge strain
9. [redacted] to determine the presence of the challenge strain in the [redacted]
10. [redacted] to determine the presence of the challenge strain in the [redacted]
11. Urine, fecal, colostrum / milk samples [redacted] to determine the presence of the challenge strain
12. Punction of [redacted] or installation of a [redacted]
13. Punction [redacted]
14. [redacted] biopsy [redacted]
15. Pregnancy check [redacted]
16. Euthanasia

The duration of all procedures listed above will only be minutes at most. Typically, the length of the observation period after vaccination is [redacted] after each vaccination. If pregnant animals have to be followed until birth, the observation period will be longer, but in general, the total number of treatments does not increase.
The interval between vaccination and challenge infection or end of the study (in case of surrogate marker for protection) will be chosen in such a way that optimal protection is to be expected. The length of the observation period after challenge infection depends on the incubation period of the pathogen, but is

generally 1 to 4 weeks. To rule out that clinical signs are caused by an unintended co-infection, non-infected control animals may be included in a study. In addition, in models for neonatal disease in sheep and goats, ewes/goats may be required to give birth to and foster the lambs / kids but these will not be infected.

[redacted]

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

For new untested vaccine candidates it needs to be proven that they fulfil the required efficacy and safety criteria. Therefore, in initial studies small numbers of animals will be used that may not be sufficient to demonstrate statistically significant differences between treatment groups. However, with such studies it will be possible to gain an estimate of the variance between individual responses of the animals in a group at these particular observation points. This information will enable calculations to identify the minimum numbers of animals needed in the groups to give sufficient likelihood of obtaining a statistically significant result by which it can be judged that the treatments have had a real effect. In particular, the variance in the groups together with the magnitude of effect will be used in power calculations to achieve 80% power at the 95% confidence level (regarded by regulatory authorities as the standard by which such experiments should be designed).

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Studies will be performed in cattle, sheep and / or goats as appropriate. Animals of both sexes can be used for this type of animal experiment unless the study has to be performed in pregnant and / or lactating animals.

Purchase of animals: The animals will be purchased from commercial suppliers, obtained from affiliated farms or bred at [redacted] facilities.
If a certain microbial status is required, animals will be purchased from farms with the respective (certified) microbiological status and / or screened prior to inclusion in the study.
If the study design requires that the animals are vaccinated and / or infected at the [redacted] or when only [redacted] the young animals have to be [redacted] accordingly from the farm of birth to the testing facility.

Special requirements
If young animals with a specific [redacted] status are required, it is often necessary to warrant that [redacted]. As these [redacted] animals are more susceptible for intercurrent infections, they receive [redacted] treatment. With the exception of studies against [redacted] pathogens, [redacted] can be given to the animals from 2 days after birth onwards as an aid in the prevention of [redacted]

Age of animals:
Age of the animals for vaccine research varies from a few hours old to adult. The age of the animals to

be used should be the minimal age recommended for use of the vaccine.
If very young lambs or kids have to be vaccinated, it might be necessary to include the ewes / goats to foster the lambs / kids. For vaccines intended to be used for pregnant / lactating animals, it may be necessary to include dams in one or more specific trimester of pregnancy, depending on the vaccination schedule to be recommended. Samples might have to been taken from the offspring, for certain studies, euthanasia of the offspring might be required.

The tables below, specify, which models would be used for the different pathogens that might be included in research projects over the next 5 years. The lists are more extensive than the actual portfolio will be, but it is not possible at this moment to predict, which pathogens will be worked on.
The animal categories listed are the age groups considered to be most sensitive and therefore have to be used to perform the basic efficacy studies.

Priorities within the R&D portfolio are based on market needs and the estimated likelihood of success of obtaining a vaccine candidate that fulfills the required product profile. Priorities can shift upon identification of new unmet needs in the field. For example, if a new [redacted] pathogen with substantial impact on the ruminant industry is discovered, this will be given priority over research into a second generation improved product for a [redacted] pathogen.

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 2 wk
New pathogen	Cattle <6 Months old	Severe (max 70%)[1]	Max 2 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 2 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 2 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 50%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
New pathogen	Cattle <6 Months old	Moderate (max 100%)[2]	Max 2 wk

[1]Vaccines are given to [redacted] in order to induce [redacted]. This part of the study does only cause mild discomfort. The information given in the table above relates to the discomfort during challenge studies in calves in [redacted] will be determined.

[2]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 50%)	Max 2 wk
[redacted]	Adult cows	Moderate (max 50%)	Max 2 wk
[redacted]	Adult cows	Mild	Max 2 wk
[redacted]	Adult cows	Mild	Max 2 wk
[redacted]	Adult ewes	Mild	Max 2 wk
[redacted]	Adult ewes	Mild	Max 2 wk
New pathogen	Adult cows	Moderate (max 100%)[1]	Max 2 wk
New pathogen	Adult ewes	Moderate (max 100%)[1]	Max 2 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
New pathogen	Adult cows	Severe (max 100%)[1]	Max 2 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle<6Months and adult cows	Moderate (max 50%)	Max 2 wk

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	sheep <6 Months	Severe (max 40%)	Max 1-2 d
	cattle<6 Months	Moderate (max 70%)	Max 2 wk
[redacted]	sheep <6 Months	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 70%)	Max 1-2 days
[redacted]	sheep <6 Months	Severe (max 50%)	Max 1 wk
[redacted]	cattle<6 Months	Moderate (max 70%)	Max 1 wk
[redacted]	sheep <6 Months	Mild	Max 2 wk
	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Severe (max 50%)	Max 1-2 d
[redacted]	sheep < 6 Months	Severe (max 50%)	Max 1-2 d
[redacted]	cattle<6 Months sheep < 6 Months	Mild	Max 2 wk
New pathogens and new types of models [redacted]. [redacted])	cattle<6 Months sheep < 6 Months	Severe (max 100%)[2]	Max 1 w

[1] Efficacy of [redacted] vaccines in ruminants is tested by [redacted] only

[2]: Studies with potential new pathogens are given the maximum discomfort score until the severity of clinical signs has been established

Based on the experience over the last 5 years and the current R&D program and priorities, the total expected number of cattle, sheep and goat per age group and discomfort category is the following:

Species	Discomfort score*	Animals <6 Months	Adult**

Cattle	Mild	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]
Sheep	Mild	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]
Goat	Mild	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]

*: Discomfort due to disease
**: [redacted]
***: Due to repeated procedures the overall discomfort will be moderate in at most 50 % of the animals [redacted]

In the vaccination studies, one or more vaccinated groups are compared to an unvaccinated control group. The group size is dependent on the challenge model and the age of the animal at the time of challenge, as for some pathogens the susceptibility for infection/disease will diminish with age, making the use of relatively large group sizes necessary to be able to show a statistically significant difference. In general, the group size used will be [redacted], but could be larger depending on the expected variation in the infection model.
The expected numbers per category of vaccine are:

Per Model	Cattle <6Months	Adult cattle	Sheep <6Months	Adult sheep	Goat <6Months	Adult goat
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

For the vaccines that are currently in the research phase, challenge will still have to be performed in [redacted] of the models to prove efficacy because of the absence of an immunological correlate of protection.

C. Re-use

Will the animals be re-used?

☐ No, continue with question D.

X Yes > Explain why re-use is considered acceptable for this animal procedure.

Re-use might be considered for animals that experience only minor discomfort during the first study, for example uninfected control animals. This approach is considered acceptable especially in case of animals that were raised under special conditions (e.g. [redacted]) to achieve a certain [redacted] status.

Are the previous or proposed animal procedures classified as 'severe'?

xNo

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:
In accordance with international regulations, animals of the target species must be used to demonstrate the safety and efficacy of a vaccine because there are no suitable alternatives or models for the induction of immunity in a whole organism or for the infection of living tissues as complex as those found in the whole animal in which the vaccines are intended to have efficacy. Therefore, during the research phase, candidate vaccines have to be tested in the same models.

Reduction: All studies are performed with the lowest possible number of animals that are required to enable meaningful interpretation of the results. This will be achieved through an ongoing evaluation of the observations in each study. The number of animals per study will be substantiated in each study protocol. According to internal procedures, the study protocol will be reviewed by the Animal Welfare Body and a statistician.

Refinement:
Where possible it is pursued to refine the routes of administration of substances and sampling techniques to improve animal welfare/to reduce discomfort of administration, but without endangering the scientific outcome. Ruminants are the target species and there are no other less innervated/sentient species that could be a model for the ruminant diseases that are studied. See next paragraph for other refinement methods that are applied.
The classic method to prove protection of a new vaccine is efficacy in a vaccination-challenge test. However, if immunological correlates of protection (e.g. a serological response) can be used to prove efficacy this will be used rather than challenge infection. When an infection model has to be used, humane endpoints will be employed and staff will be fully trained to recognize animals that experience discomfort. Animals will be closely monitored and additional health checks are performed to ensure that no animal is left suffering.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

For monitoring of the clinical health status of animals, all study animals will be checked at least once a day by a certified person. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.
Furthermore efforts are made to optimally enrich the environment during containment.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

The vaccines in the companys R&D program are unique and proprietary to the company. To show that vaccines are compatible (combined or associated use), a number of the safety and efficacy studies done with the individual products has to be repeated with the vaccines administered together according to international regulations and guidelines.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[] No

X Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

The animals are housed socially, but animals might have to be housed (temporarily) individually without physical contact (but in the same holding room) in order to prevent unintended spreading of the vaccine strain or field infection. Some studies may require limited bedding during containment. In a few cases [redacted] it is necessary to use no bedding because of scientific reasons.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

XYes > Will anaesthesia, analgesia or other pain relieving methods be used?

[] No > Justify why pain relieving methods will not be used.

Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Vaccination, injections (for application of challenge material, injection with [redacted] drugs or agents) as well as sampling of blood are part of normal farm practice/veterinary care and will induce only mild discomfort. If the sampling is repeated [redacted], the discomfort is considered to be moderate as a result of the stress when restrained and during handling of the animal.
All biotechnical procedures such as vaccination and blood sampling procedures have been described in Standard Operating Procedures (SOPs) and only well trained personnel will be responsible for the execution (GLP accredited procedures).

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Measurement of body temperatures and body weight, sampling urine, feaces, colostrum/milk, sampling on different mucosae [redacted] as well as [redacted] are part of normal farm practice/veterinary care and will induce only mild discomfort. If the following proceudres [redacted] sampling or [redacted], punction of [redacted] punction [redacted] biopsy) are repeated the discomfort is considered to be moderate.
All biotechnical procedures such as vaccination and sampling procedures have been described in Standard Operating Procedures (SOPs) and only well trained personnel will be responsible for the execution (GLP accredited procedures).
Colostrum deprived animals are more susceptible to disease and might therefore encounter discomfort related to intercurrent diseases. Transport of the animals to the testing facility might cause transient discomfort for the duration of the transport, especially for animals that are transported below the age of 10 days.
Vaccination can cause a transient increase in rectal temperature, sometimes accompanied with a reduced level of activity, and a transient vaccination site reaction. Systemic reactions generally disappear within 24 hours, but local reactions (that are generally painless) can persist for several days and even weeks.
The safety and efficacy profile of the vaccine compositions used in the research phase is not yet

determined. Therefore, it is possible that adverse events occur or that protection of the vaccinated animals is very limited.
Depending on the nature of the challenge inoculum the discomfort of the challenge can range from mild in the absence of any clinical signs (e.g. [redacted]) to moderate (e.g [redacted]) and severe (e.g. [redacted]). Vaccination will result in a significant reduction of clinical abnormalities after challenge compared to the unvaccinated control group and thus to a reduction of animals with discomfort.
In case of challenge studies with pathogens that do not cause clinical disease or only very mild disease, it might be necessary to [redacted] the animals. These procedures and any effects related to the [redacted] treatment are already taken into consideration for the discomfort levels and durations listed above in the respective tables.
The clinical health status of all animals is checked at least once a day by qualified personnel. Special attention is paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.
In consultation with the veterinarian and Study Director, if treatment does not interfere with the test results, it will be decided whether to apply adequate veterinary care including analgesia to alleviate treatment related pain (for example infection studies with [redacted] or [redacted] or pain not related to the treatment. In case of severe suffering, humane endpoints are applicable. General humane endpoints are described in an SOP (e.g. the condition of the animal prevents it from eating and drinking regularly, severe loss of body weight) and test specific humane endpoints are given in each study protocol if applicable.

Explain why these effects may emerge.

These procedures may be part of the experimental design. For vaccines intended for use in young animals, the study design may require that the animals are vaccinated and / or infected [redacted] or when only [redacted]. In these cases, the young animals have to be [redacted] accordingly from the farm of birth to the testing facility.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

All biotechnical procedures will only be performed according to standard procedures described in SOPs (GLP accredited procedures).
The number of samplings will be done in accordance with the respective guidelines or if no requirements are given, the number of samplings is reduced to a minimum number required to for a valid evaluation of results.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

To determine the efficacy of a vaccine it is necessary to challenge animals with the pathogenic organism. The severity of discomfort is depending on the nature of the pathogen (see 3.1 of the project proposal for specific clinical signs of the pathogens involved). However, the duration of severe discomfort will be limited due to the application of a humane endpoint if needed.
General humane endpoints are applicable to all animals, irrespectively of the type of experiment.
General Humane Endpoints:

- The animal experiences more than minor additional discomfort as a consequence of conditions resulting in long term or non-reversible inability to eat and or drink autonomously, fast or long lasting loss of weight, diseases or conditions that cause severe pain, suffering or discomfort such as bone fractions, force unnatural positioning and / or movements, open wounds or absesses.
- Scientific endpoints: The target of the study reached / all planned samplings have been performed
- (Reliable and useful) results cannot be reached for reasons unrelated to the study

Specific humane endpoints after infection with bovine pathogens

- In general, disease specific clinical signs (for example [redacted] or [redacted]) do not normally lead to humane endpoints, but they may affect the general health of the animal (i.e.

high fever, dullness, anorexia) leading to humane endpoints if
    - severe general clinical signs last for at least 2 days or the body temperature drops rapidly
    - the animal is unable to stand up and eat/dring actively for more than 1 day
- In [redacted] are humane endpoints.

These test-specific humane endpoints need to be described in the corresponding study protocol. Each study protocol is reviewed by the AWB before execution of the study.
In case it is difficult to reach a decision based on the pre-defined criteria for an endpoint the designated veterinarian is empowered to decide that a humane endpoint is applied/reached.

Indicate the likely incidence.

Considering the expected number of studies with the different pathogens, the expected number of animals included in the different treatment groups (i.e. vaccinated / infected / control group) and the expected severity, at most 20 % of the animals is expected to have severe discomfort that would require euthanasia.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

For studies without challenge, discomfort will be mild to moderate depending on the number of sampling points. For vaccination-challenge studies, the type and severity of the clinical signs are depending on the type of challenge infection. Similar to natural field infections they may cause mild to severe pain, distress, suffering or even impending death. See B for an overview of the different pathogens involved, the maximal discomfort caused by the disease and the maximum number of animals expected to reach the highest discomfort category. Vaccination is expected to reduce the level of discomfort after challenge, but the non-vaccinated control group will experience the symptoms of the natural infection.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Postmortem investigation can be part of the experimental design to evaluate (histo)pathological lesions at the injection sites and or to evaluate effect of the infection on different organ systems and or to attempt re-isolation of the inoculum from tissues and organs.
In addition, animals vaccinated with a non-licensed vaccine or infected with a pathogen cannot be returned to the farm of origin or transported to another farm to prevent the spread of disease. Therefore, all animals might have to be euthanized at the end of the study or when a humane endpoint is reached. Control animals that have not been vaccinated and infected may be reused or returned to the farm of origin or transported to another farm. In case of a surrogate immunological marker (no challenge), animals housed on contract farms can remain on the farm or transported to other farms until the end of their natural/economic life.
Moreover, return of the animals to commercial farms or slaughter for human consumption is often prohibited by the current legislation on use of antibiotics.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

Centrale Commissie Dierproeven

14.

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	22100
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 3 Type of animal procedure: Research: Assay development and preparation of biomaterials

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

The potency of each inactivated vaccine batch used in development studies has to be determined to set the limits for release and to determine the stability. Traditionally, the potency of inactivated vaccines, is measured in a so-called *in vivo* potency in [redacted]. During the research phase of a vaccine project, the test protocol for the potency test is determined. In an initial study, the strenghth of the immune reponse is compared between the different animal species. In further studies, it is investigated, whether the antibody response is dose dependent and the release criteria are determined.
Apart from the replacement of experimental animals, *in vitro* potency tests are very much preferred over *in vivo* testing for multiple other reasons including costs and timelines. However, for several inactivated vaccines, *in vivo* potency tests are still necessary because it is either not possible to determine the antigen and/or adjuvant content *in vitro* or an *in vivo* batch test is mandatory under the respective legislation (e.g. Ph.Eur monograph.).
In such a batch potency test, animals are injected with one or more doses of the vaccine batch. The potency is preferably determined by measuring the antibody response. In a few cases, determination the protection against challenge infection is mandatory or necessary for scientific / technical reasons.
Biomaterials such as specific antisera or monoclonal antibodies are needed in most vaccine projects for different purposes such as the identification, characterisation and quantification of the vaccine strain, neutralizing the vaccine virus, setting up *in vitro* tests et cetera.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Two or more of the following treatments will be employed in order to fulfil the requirements for the potency studies as laid down in the Ph Eur *(in italics the frequency of the treatments).* The same

treatments are also applied for the preparation of biomaterials*:*
1. Blood sampling [redacted] to determine [redacted]
2. Test substance administration [redacted]
3. Weighing [redacted]
4. Euthanasia
The duration of these procedures will only be minutes.
In case of potency tests that involve challenge infection, the following additional treatments are employed
5. Administration of challenge material [redacted]
6. Clinical observation ([redacted] *daily for up to* [redacted] *days*)

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

The group sizes for potency tests that are specified in guidelines typically range between 5 and 10. For those models that are not described in specific regulations, a group size of 5-10 animals is generally accepted by regulatory authorities. If sufficient knowledge on the variation is available the group size will be calculated such that a statistically significant difference between standard and substandard vaccine batches can be made with 80% power and 95% confidence.
For the preparation of antisera the minimal number of animals that will provide the required volume will be used.
For [redacted] mice will be used per antigen.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

For some vaccines, the species to be used for a batch potency test is prescribed in a Ph.Eur monograph.
In general, [redacted] are preferred over the ruminant target species because they are more genetically homogeneous and better microbiologically controlled.
Therefore, less variance in response and better reproducibility can be achieved, which means that the number of experimental animals can be lower than when using ruminants.
For some vaccines, the type of laboratory animals to be used for a batch potency test is prescribed in a Ph.Eur monograph.
Preparation of antisera in non-ruminant animals or murine monoclonal antibodies has the advantage, that these animals are free of antibodies against other ruminant pathogens.
Based on the experience over the last 5 years and the current R&D program and priorities, the total expected number of laboratory animals is the following:
[redacted]
[redacted] of both sexes will be used, but for [redacted] only females will be included in studies because of a higher risk of fighting in male animals and the relatively long time that the animals are in an experiment. With regard to the length of these experiments ([redacted]) it is not acceptable to use males, because they would need to be single housed.
Welfare concerns are the basis for the preferred use of female [redacted] because they hardly fight. Data from recently executed experiments with male animals have shown that in half of the experiments there was a loss of animals because of severe fighting. This has resulted in the repetition of these experiments and therefore in the use of more animals. A loss of animals due to fighting has never occurred when female animals were used. We consider the aggression and fighting a worrying impairment of welfare. Moreover, the aggression will cause stress, which is known to have effects on the immune system.
In addition, use of animals of both sexes would increase the variablity and thereby increase the number of animals needed.
In these studies the functioning of the immune system is crucial and variation in the immune response

caused by external factors should be avoided as much as possible.

Origin [redacted]
All animals are supplied by certified vendors accompanied by a health certificate according to FELASA recommendations. All purchased animals have a SPF status.

Origin [redacted]
Own SPF [redacted] breeding unit or commercial vendor.

Age of animals:
The required species and age (or weight) is usually designated as the most sensitive species or age for the test component in question. If such specific knowledge is not available the most practical choices are made, based on possibilities for purchase and housing conditions. Moreover, animals must be immunologically fit to be subjected to immunizations and blood samplings.

C. Re-use

Will the animals be re-used?

X No, continue with question D.

Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

XNo

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:
Apart from the replacement of experimental animals, *in vitro* potency tests are very much preferred over *in vivo* testing for multiple other reasons including costs and timelines. Therefore, multidisciplinary teams are active at the company to replace *in vivo* potency tests by *in vitro* tests such as antigenic mass assays for the potency testing of vaccines.

Reduction: The animal species that is expected to give the most discriminatory test with the smallest number of animals will be used. The number of animals per study will be substantiated in each study protocol. Each study protocol will be reviewed by the Animal Welfare Body and a statistician.

Refinement: Following the codes of practice for immunization is the basis for refinement in these animal procedures. Where possible it is pursued to refine the routes of administration of substances and sampling techniques to improve, but without endangering the scientific outcome. For example; blood sampling in rodents is done under anesthesia. See next paragraph for other refinement methods that are applied.
Where ever possible, potency testing will be based on the testing of antibodies or other correlates of protection rather than by challenge.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Following the code of practice for immunization and the code of practice for monitoring the welfare of the animals is the basis for refinement in these animal procedures.
In general animals are always housed socially, but animals might have to be (temporarily) separated due to fighting or because of veterinary concerns. Furthermore, to enhance animal welfare, species specific environmental enrichment is provided to all animals.

For monitoring of the clinical health status of animals, all study animals will be checked at least once a day by a certified person. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

A potency test model needs to be developed for a specific vaccine. Experiences from vaccines with similar composition can be used, but eventually, the model has to be validated for the specific vaccine. Specific biomaterials have to be available for each project. Prior to the preparation of new biomaterials, the scintific literature and the company database for biomaterials available at the different research sites will be consulted in order to prevent unnecessary preparation of new biomaterials.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[] No

X Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

Some studies may require limited bedding during containment.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

XYes > Will anaesthesia, analgesia or other pain relieving methods be used?

X No > Justify why pain relieving methods will not be used.

X Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Injections (vaccination, inoculation of challenge material) and blood sampling are part of normal veterinary care / commonly used biotechnical procedures and will induce only mild discomfort or moderate discomfort of very short duration.

Anesthesia will be applied during blood sampling of [redacted]. Anesthesia will not be applied during sampling blood from [redacted]. In these species blood sampling causes only

mild discomfort or moderate discomfort of very short duration. Applying anesthesia would only increase discomfort as the animal needs to be restrained during administration.

For each species blood sampling and other biotechnical procedures have been described in Standard Operating Procedures (SOPs) (GLP accredited procedures).
As the vaccines to be tested have already been found to be safe in the target species (ruminants) it is very unlikely that they will cause adverse effects in non-target animals.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

In general, biotechnological procedures such as weighing and sampling may result in discomfort, because animals need to be fixated and especially if performed repeatedly.
Moreover, application of the vaccine can result in a transient increase in rectal temperature, sometimes accompanied with a reduced level of activity, and a transient vaccination site reaction. Systemic reactions will generally disappear within 24 hours, but local reactions (swelling redness), that are generally painless, can persist for several days and even weeks, but these local reactions do not affect normal behaviour (activity, feeding and drinking).
Depending on the nature of the challenge inoculum, the discomfort of the challenge can range from mild in the absence of any clinical signs [redacted]) to moderate [redacted] and severe ([redacted] Vaccination will result in a significant reduction of clinical abnormalities after challenge compared to the unvaccinated control group.
For monitoring of the clinical health status of animals, all study animals will be checked at least once a day by a certified person. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.
In case animals experience discomfort (whether or not related to the treatment), it will be decided in consultation with the veterinarian and Study Director whether to apply adequate veterinary care to alleviate unexpected pain and/or distress (if treatment does not interfere with the test results). In case of severe suffering, humane endpoints are applicable. General humane endpoints are described in an SOP (e.g. the condition of the animal prevents it from eating and drinking regularly, severe loss of body weight) and test specific humane endpoints are given in each study protocol if applicable.

Explain why these effects may emerge.

These procedures and if applicable the clinical signs caused by the challenge may be part of the experimental design.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Weighing is part of normal veterinary care and will induce only mild discomfort. Taking samples of mucosal surfaces or urine will result in mild discomfort, with the exception of repeated mucosal swabbing, which is considered to be moderate discomfort. Biotechnical procedures have been described in SOPs (GLP accredited procedures). Unless required by the applicable regulations or scientific reasons, potency testing will be based on the testing of correlates of protection such as antibody levels rather than by challenge.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

In potency tests that require challenge with a pathogenic organism, the severity of discomfort is depending on the nature of the pathogen. However, the duration of severe discomfort will be limited due to the application of a humane endpoint if needed.
General humane endpoints are applicable to all animals, irrespectively of the type of experiment.
General Humane Endpoints:

- The animal experiences more than minor additional discomfort as a consequence of conditions resulting in long term or non-reversible inability to eat and or drink autonomously, fast or long lasting loss of weight, diseases or conditions that cause severe pain, suffering or discomfort such

as bone fractions, force unnatural positioning and / or movements, open wounds or absesses.
- Scientific endpoints: The target of the study reached / all planned samplings have been performed
- (Reliable and useful) results cannot be reached for reasons unrelated to the study

Specific humane endpoints after infection with bovine pathogens

- In general, disease specific clinical signs ([redacted] do not normally lead to humane endpoints, but they may affect the general health of the animal (i.e. high fever, dullness, anorexia) leading to humane endpoints if
  - severe general clinical signs last for at least 2 days or the body temperature drops rapidly
  - the animal is unable to stand up and eat/dring actively for more than 1 day

These test-specific humane endpoints are described in the corresponding study protocol. Each study protocol is reviewed by the AWB before execution of the study.
In case it is difficult to reach a decision based on the pre-defined criteria for an endpoint the designated veterinarian is empowered to decide that a humane endpoint is applied/reached.

Indicate the likely incidence.

As the overall majority of potency tests as well as the preparation of biomaterials do not require challenge, the incidence of severe discomfort requiring euthanasia is estimated to be less than 2 %.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Discomfort will be mild (≥75%) to moderate (≤25%) depending on the number of sampling points, type of biotechnical procedure and whether the challenge infection causes disease in the model animal.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

For some potency models it is necessary to investigate the presence of (histo)pathological lesions and take organ and tissue samples for re-isolation of the vaccine strain.
For the preparation of [redacted] it is necessary to kill the animals in order to obtain the required volumes.
For preparation of [redacted]

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

15.

Geachte CCD,

Bijgevoegd zijn de antwoorden op gestelde vragen vanuit CCD n.a.v. brief 6 juni 2017.

Uw referentie is: Aanvraagnummer AVD2210020171629 (Research on new ruminant vaccines) [redacted]

In paars zijn de antwoorden weergegeven. Naar aanleiding van deze vragen zijn ook de documenten Bijlagen beschrijving dierproeven aangepast. Ook hierin zijn de aanpassingen met paarse tekst weergegeven.

We hopen u hiermee voldoende geïnformeerd te hebben. Indien er nog vragen zijn, dan vernemen wij deze graag.

Met vriendelijke groeten,

[redacted]

[redacted]

[redacted],
In aanvulling op de eerder gestelde vragen, hebben wij nog enkele vragen over aanvragen AVD2210020171629 en [redacted].

[redacted] *dieren:*
In Bijlage Dierproeven 3.4.4.1 en 3.4.4.2 van aanvraag AVD2210020171629 en in Bijlage Dierproeven [redacted] worden ook [redacted] dieren en [redacted] genoemd. In de Wet op de dierproeven staat dat deze wet ook van toepassing is op [redacted] van zoogdieren [redacted] te ondervinden.

Zijn de [redacted] dieren meegenomen in de [redacted]
Kunt u een inschatting maken om hoeveel [redacted] het zal gaan, per Bijlage Dierproeven? Is hierbij ook rekening gehouden met het ongerief van de ongeboren dieren?
Indien de [redacted] dieren niet meegenomen zijn in de aantallen, kunt u dan nieuwe Bijlagen Dierproeven sturen, waarin deze aantallen zijn verwerkt? Anders kunt u de overige vragen (aantal dieren en ongerief) beantwoorden zonder nieuwe Bijlagen Dierproeven. –
De aantallen en het ongerief waren al meegenomen, echter stonden de [redacted] dieren als "cattle / sheep <6 maanden" vernoemd bij de [redacted] modellen. Dit is in de nieuwe Bijlage Dierproeven 3.4.4.1 en 3.4.4.2 van aanvraag AVD2210020171629 [redacted] aangepast. De betreffende aantallen zijn nu in de regel [redacted] weergegeven.

Bedding:
In Bijlage Dierproeven 3.4.4.1 en 3.4.4.2 van aanvraag AVD2210020171629 [redacted] wordt aangegeven dat er geen bedding wordt toegepast. Kunt u onderbouwen waarom het gebruik van bedding niet mogelijk is?
In de dierproeven genoemd in Bijlage Dierproeven [redacted] is beperkt aanbod van bedding mogelijk. De onderbouwing voor het niet gebuiken van bedding in de studies genoemd in Bijlage Dierproeven 3.4.4.1 en 3.4.4.2 van aanvraag AVD2210020171629 [redacted] is gegeven in de begeleidende brief dd 23 mei j.l. De volgende zin is ook toegevoegd in de betreffende bijlages: "In [redacted] infection models it is necessary to collect the [redacted] in order to perform a visual inspection and to determine the [redacted] and / or total amount of [redacted] Therefore, no substrate can be offered in this type of studies."

Challenge:
Kunt u voor Bijlage Dierproeven 3.4.4.3 van aanvraag AVD2210020171629 en voor Bijlage Dierproeven [redacted] aangeven hoeveel dieren een challenge zullen ondergaan?
Minder dan [redacted] van de dieren zullen een challenge ondergaan. De zin "Challenge infection will be performed in [redacted] of the animals." is toegevoegd bij vraag K in voor Bijlage Dierproeven 3.4.4.3 van aanvraag AVD2210020171629 [redacted]

[redacted]

Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze e-mail op. Mocht u langere tijd nodig hebben om de vragen te beantwoorden, wilt u dit dan aangeven?
De behandeling van uw aanvraag wordt opgeschort tot het moment dat wij de aanvullende informatie hebben ontvangen. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn ontvangen; [redacted]

Als er nog vragen zijn, dan hoor ik dat graag.

Met vriendelijke groeten,
Namens de Centrale Commissie Dierproeven

[redacted]
www.centralecommissiedierproeven.nl
Postbus 20401 | 2500 EK | Den Haag
T: 0900 – 28 000 28 (10 ct/min)
E: info@zbo-ccd.nl

Centrale Commissie Dierproeven

16.

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	22100
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 1 — Type of animal procedure: Research: Infection studies in ruminants

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

Infection studies will be performed for one of the three different reasons:

1) To determine the pathogenicity of new pathogens (to prove Koch's postulates), variants of known pathogens, should they appear or the role of specific genes in the pathogenicity and interaction with the host's immune system.
2) To develop an infection model that will be used in vaccination-infection studies to assess the efficacy of vaccine candidates (see Addendum 2).
3) To assess the safety profile of a live attenuated vaccine candidate obtained by "classical" means (e.g. in vitro passage or chemical mutagenesis) or gene-modification (including vector vaccins).

In general, the application of a pathogen will be done via the natural route of infection, but if the natural route does not induce all presentations of a disease under laboratory conditions, it might be necessary to use another route (e.g. parenteral injection to induce a systemic infection).
Application of a potential vaccine candidate will typically be done by the route intended as application route of the future product, but in specific cases it might be necessary to follow the route that gives highest risk of adverse events in order to make a meaningful assessment of the safety profile.
In case a pathogen or vaccine candidate might cause transplacental infection, pregnant animals at one or more specified stage of pregnancy have to be used in the infection studies. The animals are then observed until the end of pregnancy to determine the outcome of the pregnancy and the health status of the offspring. Samples might have to be taken from the offspring to determine the presence of the pathogen / vaccine candidate or specific pre-colostral antibodies. [redacted]

After inoculation of the vaccine candidate or pathogen, one or more of the following parameters will be

evaluated:

- Clinical signs (e.g. changes in general health and or disease specific symptoms and local reactions)
- Body temperature (rectal temperature)
- Body weight
- Virus, bacterial or parasites shedding [redacted] mucosal surfaces, sampling of faeces, urine, milk), [redacted]
- Viraemia, bacteraemia or parasitemia or haematological parameters (blood/ [redacted])
- Post mortem examination (macroscopical and microscopical)

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Four or more of the following procedures will be undertaken depending on the characteristics of the vaccine/ pathogen involved; the types of pathogens are described under B (in italics the frequency of the procedures):

1. Daily observation / scoring clinical signs including measurement of rectal temperature ([redacted]
2. Weighing [redacted]
3. Blood sampling [redacted] to determine [redacted] parameters and / or to determine the presence of the pathogen or vaccine candidate in the blood
4. [redacted] treatment by application of [redacted] and / or inoculation of [redacted]
5. Administration of pathogen or vaccine candidate [redacted]
6. Swabbing of (mucosal) surfaces [redacted] to determine the excretion of the pathogen or vaccine candidate
7. [redacted] to determine the presence of the pathogen or vaccine candidate in [redacted]
8. [redacted] to determine the presence of the pathogen or vaccine candidate in the [redacted]
9. Urine, fecal, colostrum / milk samples [redacted] to determine the presence of the pathogen or vaccine candidate
10. Punction of [redacted] or installation of a [redacted]
11. Punction [redacted]
12. [redacted] biopsy [redacted]
13. Pregnancy check [redacted]
14. Euthanasia

The duration of all procedures listed above will only be minutes at most. Typically, the length of the observation period after infection is [redacted]. If pregnant animals have to be followed until birth, the observation period will be longer, but in general, the total number of treatments does not increase.
To rule out that clinical signs are caused by an unintended co-infection, non-infected control animals may be included in a study. In addition, in models for neonatal disease in sheep and goats, ewes/goats may be required to give birth to and foster the lambs / kids but these will not be infected.

[redacted]

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

In this type of initial studies, it is not obligatory to demonstrate statistically significant differences between treatment groups. However, these studies will enable an estimation of the variance between individual responses of the animals in a group at these particular observation points. Based on this information the minimum numbers of animals per group needed to demonstrate the efficacy of a vaccine during the development phase can be estimated. In this type of experiments, the group size is in general 5-8 animals, depending on the expected variation in the infection model. This group size is in line with the group size for infection studies as specified in most European Pharmacopoeia monographs on ruminant vaccines.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Studies will be performed in cattle, sheep and / or goats as appropriate. Animals of both sexes can be used for this type of animal experiment unless the study has to be performed in pregnant and / or lactating animals.

Purchase of animals: The animals will be purchased from commercial suppliers, obtained from affiliated farms or bred at [redacted] facilities.
If a certain microbial status is required, animals will be purchased from farms with the respective (certified) microbiological status and / or screened prior to inclusion in the study.
If the study design requires that animals are vaccinated and / or infected at [redacted] or when only a [redacted], the young animals have to be [redacted] accordingly from the farms of birth to the testing facility.

Special requirements
If young animals with a specific antibody status are required, it is often necessary to warrant that [redacted]. As these [redacted] animals are more susceptible for intercurrent infections, they receive [redacted]. With the exception of studies against [redacted] pathogens, colostrum can be given to the animals from 2 days after birth onwards as an aid in the prevention of [redacted]

Age of animals:
Age of the animals for vaccine research varies from a few hours old to adult. The age of the animals to be used should be the age at which clinical disease is expected or the minimal age recommended for use of the vaccine.
If the infection has to be done in very young lambs or kids, it might be necessary to include the ewes / goats to foster the lambs / kids. In order to study transplacental spread of the pathogen or vaccine

candidate it may be necessary to include dams in one or more specific trimester of pregnancy. Samples might have to been taken from the offspring, for certain studies, euthanasia of the offspring might be required.

The tables below specify which models would be used for the different pathogens that might be included in infection studies during the research projects over the next 5 years. The lists are more extensive than the actual portfolio will be, but it is not possible at this moment to predict exactly which pathogens will be worked on.
The animal categories listed are the age groups considered to be most sensitive and therefore have to be used to perform the basic efficacy and safety studies.

Priorities within the R&D portfolio are based on market needs and the estimated likelihood of success of obtaining a vaccine candidate that fulfills the required product profile. Priorities can shift upon identification of new unmet needs in the field. For example, if a new [redacted] pathogen with substantial impact on the ruminant industry is discovered, this will be given priority over research into a second generation improved product for a [redacted] pathogen.

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 2 wk
New pathogen	Cattle <6 Months old	Severe (max 70%)[1]	Max 2 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 2 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 2 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 50%)	Max ± 2 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
New pathogen	Cattle <6 Months old	Moderate (max 100%)[2]	Max 2 wk

[1]Vaccines are given to [redacted] in order to [redacted]. This part of the study does only cause mild discomfort. The information given in the table above relates to the discomfort during challenge studies [redacted] will be determined.

[2]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 50%)	Max 2 wk
[redacted]	Adult cows	Moderate (max 50%)	Max 2 wk
[redacted]	Adult cows	Mild	Max 2 wk
[redacted]	Adult cows	Mild	Max 2 wk
[redacted]	Adult ewes	Mild	Max 2 wk
[redacted]	Adult ewes	Mild	Max 2 wk
New pathogen	Adult cows	Moderate (max 100%)[1]	Max 2 wk
New pathogen	Adult ewes	Moderate (max 100%)[1]	Max 2 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
New pathogen	Adult cows	Severe (max 100%)[1]	Max 1 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle <6 Months and adult cows	Moderate (max 50%)	Max 2 wk

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	sheep <6 Months	Severe (max 40%)	Max 1-2 d
	cattle<6 Months	Moderate (max 70%)	Max 2 wk
[redacted]	sheep <6 Months	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 70%)	Max 1-2 days
[redacted]	sheep <6 Months	Severe (max 50%)	Max 1 wk
[redacted]	cattle<6 Months	Moderate (max 70%)	Max 1 wk
[redacted]	sheep <6 Months	Mild	Max 2 wk
	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Severe (max 50%)	Max 1-2 d
[redacted]	sheep < 6 Months	Severe (max 50%)	Max 1-2 d
[redacted]	cattle<6 Months sheep < 6 Months	Mild	Max 2 wk
New pathogens and new types of models ([redacted]	cattle<6 Months sheep < 6 Months	Severe (max 100%)[2]	Max 1 w

[1] Efficacy of [redacted] vaccines in ruminants is tested by [redacted] only

[2]: Studies with potential new pathogens are given the maximum discomfort score until the severity of clinical signs has been established

Based on the experience over the last 5 years and the current R&D program and priorities, the total expected number of cattle, sheep and goat per age group and discomfort category is the following:

Species	Discomfort score*	Animals<6 months	Adult**
Cattle	Mild	[redacted]	[redacted]

	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]
Sheep	Mild	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]
Goat	Mild	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]

*: Discomfort due to disease
**: Including [redacted]
***: Due to repeated procedures the overall discomfort will be moderate in at most 50% of the animals ([redacted] [redacted]

In the infection studies, one or more groups are compared to an uninfected control group. The group size is dependent on the disease model. In general, the group size used will be [redacted] but could be larger depending on the expected variation in the infection model. The expected numbers per category of vaccine are:

Per Model	Cattle <6Months	Adult cattle	Sheep <6Months	Adult sheep	Goat <6Months	Adult goat
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

C. Re-use

Will the animals be re-used?

☐ No, continue with question D.

X Yes > Explain why re-use is considered acceptable for this animal procedure.

Re-use might be considered for animals that experience only minor discomfort during the first study, for example uninfected control animals. This approach is considered acceptable especially in case of animals that were raised under special conditions (e.g. [redacted]) to achieve a certain [redacted] status

Are the previous or proposed animal procedures classified as 'severe'?

xNo

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research

strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:
In accordance with international regulations, animals of the target species must be used to demonstrate the safety and efficacy of a vaccine because there are no suitable alternatives or models for the induction of immunity in a whole organism or for the infection of living tissues as complex as those found in the whole animal in which the vaccines are intended to have efficacy. Therefore, during the research phase, infection models have to be developed in the target animals-and candidate vaccines have to be tested in the same models.

Reduction: All studies are performed with the lowest possible number of animals that are required to enable meaningful interpretation of the results. This will be achieved through an ongoing evaluation of the observations in each study. The number of animals per study will be substantiated in each study protocol. According to internal procedures, the study protocol will be reviewed by the Animal Welfare Body and a statistician.

Refinement:
Where possible it is pursued to refine the routes of administration of substances and sampling techniques to improve animal welfare/to reduce discomfort of administration, but without endangering the scientific outcome. Ruminants are the target species and there are no other less innervated/sentient species that could be a model for the ruminant diseases that are studied. See next paragraph for other refinement methods that are applied.
The classic method to prove protection of a new vaccine is efficacy in a vaccination-challenge test. However, if immunological correlates of protection (e.g. a serological response) can be used to prove efficacy this will be used rather than challenge infection. When an infection model has to be used, humane endpoints will be employed and staff will be fully trained to recognize animals that experience discomfort. Animals will be closely monitored and additional health checks are performed to ensure that no animal is left suffering.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

For monitoring of the clinical health status of animals, all study animals will be checked at least once a day by a certified person. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.
Furthermore efforts are made to optimally enrich the environment during containment.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

The vaccines in the companys R&D program are unique and proprietary to the company. To show that vaccines are compatible (combined or associated use), a number of safety and efficacy studies done with the individual products has to be repeated with the vaccines administered together according to international regulations and guidelines.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[] No

X Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

The animals are housed socially, but animals might have to be housed (temporarily) [redacted] in order to [redacted]

[redacted]. Some studies may require limited bedding during containment. [redacted] **infection models it is necessary to collect the [redacted] in order to perform a visual inspection and to determine the [redacted] and / or total amount of [redacted] Therefore, no substrate can be offered in this type of studies.**

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

XYes > Will anaesthesia, analgesia or other pain relieving methods be used?

[] No > Justify why pain relieving methods will not be used.

Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Injections (for application of challenge material, injection with [redacted] drugs or agents) as well as sampling of blood are part of normal farm practice/veterinary care and will induce only mild discomfort. If the sampling is repeated (>5), the discomfort is considered to be moderate as a result of the stress when restrained and during handling of the animal.
All biotechnical procedures such as vaccination and blood sampling procedures have been described in Standard Operating Procedures (SOPs) (GLP accredited procedures).

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Measurement of body temperatures and body weight, sampling urine, feaces, colostrum/milk, sampling on different mucosae [redacted] as well as [redacted] and pregnancy check are part of normal farm practice/veterinary care and will induce only mild discomfort.
If the following proceudres ([redacted] sampling or [redacted], punction of [redacted] punction [redacted] biopsy) are repeated the discomfort is considered to be moderate.
All biotechnical procedures such as inoculation and sampling procedures have been described in Standard Operating Procedures (SOPs) and only well trained personnel will be responsible for the execution (GLP accredited procedures).
[redacted] animals are more susceptible to disease and might therefore encounter discomfort related to intercurrent diseases. Transport of the animals to the testing facility might cause transient discomfort for the duration determination the protection against challenge infection of the transport, especially for animals that are transported [redacted].
The pathogenticiy of the pathogen / safety profile of the vaccine candidate used in the infection studies is not yet determined. Therefore, it is possible that adverse events occur.
Depending on the nature of the pathogen / vaccine candidate the discomfort of the infection can range from mild in the absence of any clinical signs ([redacted]) to moderate [redacted] and severe ([redacted]

In case an infection model has to be developed for pathogens that do not cause clinical disease or only very mild disease, it might be necessary to [redacted] the animals. These procedures and any effects related to the [redacted] treatment are already taken into consideration for the discomfort levels and durations listed above in the respective tables.
The clinical health status of all animals is checked at least once a day by qualified personnel. Special attention is paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.
In consultation with the veterinarian and Study Director, if treatment does not interfere with the test results, it will be decided whether to apply adequate veterinary care including analgesia to alleviate treatment related pain (for example infection studies with [redacted] pathogens or [redacted] or pain not related to the treatment. In case of severe suffering, humane endpoints are applicable. General humane endpoints are described in an SOP (e.g. the condition of the animal prevents it from eating and drinking regularly, severe loss of body weight) and test specific humane endpoints are given in each study protocol if applicable.

Explain why these effects may emerge.

These procedures may be part of the experimental design. For vaccines intended for use in young animals, the study design may require that animals are vaccinated and / or infected [redacted] or when only a [redacted]. In these cases, the young animals have to be [redacted] accordingly from the farm of birth to the testing facility.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

All biotechnical procedures will only be performed according to standard procedures described in SOPs (GLP accredited procedures).
The number of samplings is reduced to a minimum number required to for a valid evaluation of results.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

The severity of discomfort is depending on the nature of the pathogen (see 3.1 of the project proposal for specific clinical signs of the pathogens involved). However, the duration of severe discomfort will be limited due to the application of a humane endpoint if needed.
General humane endpoints are applicable to all animals, irrespectively of the type of experiment.
General Humane Endpoints:

- The animal experiences more than minor additional discomfort as a consequence of conditions resulting in long term or non-reversible inability to eat and or drink autonomously, fast or long lasting loss of weight, diseases or conditions that cause severe pain, suffering or discomfort such as bone fractions, force unnatural positioning and / or movements, open wounds or absesses.
- Scientific endpoints: The target of the study reached / all planned samplings have been performed
- (Reliable and useful) results cannot be reached for reasons unrelated to the study

Specific humane endpoints after infection with bovine pathogens

- In general, disease specific clinical signs (for example [redacted] or [redacted] clinical signs) do not normally lead to humane endpoints, but they may affect the general health of the animal (i.e. high fever, dullness, anorexia) leading to humane endpoints if
  - severe general clinical signs last for at least 2 days or the body temperature drops rapidly
  - the animal is unable to stand up and eat/dring actively for more than 1 day
- In the [redacted] are humane endpoints.

These test-specific humane endpoints are described in the corresponding study protocol. Each study protocol is reviewed by the AWB before execution of the study.
In case it is difficult to reach a decision based on the pre-defined criteria for an endpoint the designated veterinarian is empowered to decide that a humane endpoint is applied/reached.

Indicate the likely incidence.

Considering the expected number of studies with the different pathogens, the expected number of animals included in the different treatment groups (i.e. infected vs control group) and the expected severity, at most 30 % of the animals is expected to have severe discomfort that would require euthanasia.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

For the infection studies the type and severity of the clinical signs are depending on the type of disease. Similar to natural field infections they may cause mild to severe pain, distress, suffering or even impending death. See B for an overview of the different pathogens involved, the maximal discomfort caused by the disease and the maximum number of animals expected to reach the highest discomfort category.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Postmortem investigation can be part of the experimental design to evaluate (histo)pathological lesions at different organ systems and or to attempt re-isolation of the inoculum from tissues and organs.
In addition, animals with a pathogen cannot be returned to the farm of origin or transported to another farm to prevent the spread of disease. Therefore, all animals might have to be euthanized at the end of the study or when a humane endpoint is reached. Control animals that have not been infected may be reused or returned to the farm of origin or transported to another farm.
Moreover, return of the animals to commercial farms or slaughter for human consumption is often prohibited by the current legislation on use of antibiotics.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	22100
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: Research: Vaccination challenge studies in ruminants

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

During the research stage of a vaccine project, studies are carried out to estimate, whether the candidate vaccine is likely to successfully pass the safety and efficacy studies that are later on performed in the development phase.
The aim of this type of vaccination studies is to test if a vaccine candidate i) has acceptable safety characteristics and ii) is efficacious i.e. induces a measurable immune response and / or protects against one or more aspects of the disease caused by the pathogen(s) involved. To this end, animals are vaccinated according to the anticipated schedule and observed for local and systemic reaction after vaccination. The immune response after vaccination is determined. In the absence of a correlate of protection, the vaccinated animals or in case the vaccine is intended for the induction of passive protection, animals fed colostrum from vaccinated mothers will be infected with a challenge strain according to the previously established infection model (see DAP 1). Unvaccinated ~~a~~animals will also be included in the experiment as control to determine the efficacy of the experimental vaccine. After vaccination and after challenge infection, one or more of the following parameters will be evaluated:

- Clinical signs (e.g. changes in general health and or disease specific symptoms and local reactions)
- Body temperature (rectal temperature)
- Body weight
- Virus, bacterial or parasites shedding (swabbing of mucosal surfaces, sampling of faeces, urine, milk), viral/bacterial/parasitic load in [redacted] or other tissues ([redacted]
- Viraemia, bacteraemia or parasitemia or haematological parameters (blood/[redacted] sampling)
- Post mortem examination (macroscopical and microscopical)

On the basis of the study results, it will be determined if a vaccine candidate has an acceptable risk-benefit

profile that is in line with criteria that have been laid down in EU directives, the Pharmacopoeia Europaea (Ph.Eur) and guidelines and regulations of the European Medicines Agency and other international regulatory bodies when applicable. Some fine-tuning of the composition (e.g. changes in antigens/adjuvant included) may be necessary before the optimal vaccine has been reached (proof of concept). In some cases it may not be possible to obtain proof of concept with the available candidates and knowledge of the pathogen, which means that the research project will be stopped.
During these orientating vaccination studies, it will also be attempted to find a serological correlate with protection or another surrogate immunological marker that will enable the drawing of conclusions on the efficacy of a candidate vaccine without challenge in further studies.
In case of [redacted] diseases, it might be necessary to mimic the [redacted] effect of [redacted] factors in order to reach the required sensitivity of the animals to the challenge infection with a [redacted] pathogen.
In case a vaccine is intended for protection against transplacental infection, pregnant animals are challenged at one or more specified stage of pregnancy. The animals are then observed until the end of pregnancy to determine the outcome of the pregnancy and the health status of the offspring. Samples might have to be taken from the offspring to determine the presence of the challenge strain or specific pre-colostral antibodies. [redacted]

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Four or more of the following procedures will be undertaken depending on the characteristics of the vaccine/ pathogen involved; the types of pathogens are described under B (in italics the frequency of the procedures):

1. Daily observation / scoring clinical signs including measurement of rectal temperature *(*[redacted]*)*
2. Weighing *(*[redacted]
3. Blood sampling [redacted] to determine [redacted] parameters and / or to determine the presence of the vaccine strain in the blood and / or to determine the presence of the challenge strain in the blood
4. Vaccine administration [redacted]
5. Palpation of the injection site [redacted]
6. [redacted] treatment by application of [redacted] and / or inoculation of [redacted]
7. Challenge administration [redacted]
8. Swabbing of (mucosal) surfaces [redacted] to determine the excretion of the vaccine and / or [redacted]) to determine the excretion of the challenge strain
9. [redacted] to determine the presence of the challenge strain in the [redacted]
10. [redacted] to determine the presence of the challenge strain in the [redacted]
11. Urine, fecal, colostrum / milk samples [redacted] to determine the presence of the challenge strain
12. Punction of [redacted] or installation of a [redacted]
13. Punction [redacted]
14. [redacted] biopsy [redacted]
15. Pregnancy check [redacted]
16. Euthanasia

The duration of all procedures listed above will only be minutes at most. Typically, the length of the observation period after vaccination is [redacted] after each vaccination. If pregnant animals have to be followed until birth, the observation period will be longer, but in general, the total number of treatments does not increase.
The interval between vaccination and challenge infection or end of the study (in case of surrogate marker for protection) will be chosen in such a way that optimal protection is to be expected. The length of the observation period after challenge infection depends on the incubation period of the pathogen, but is

generally 1 to 4 weeks. To rule out that clinical signs are caused by an unintended co-infection, non-infected control animals may be included in a study. In addition, in models for neonatal disease in sheep and goats, ewes/goats may be required to give birth to and foster the lambs / kids but these will not be infected.

[redacted]

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

For new untested vaccine candidates it needs to be proven that they fulfil the required efficacy and safety criteria. Therefore, in initial studies small numbers of animals will be used that may not be sufficient to demonstrate statistically significant differences between treatment groups. However, with such studies it will be possible to gain an estimate of the variance between individual responses of the animals in a group at these particular observation points. This information will enable calculations to identify the minimum numbers of animals needed in the groups to give sufficient likelihood of obtaining a statistically significant result by which it can be judged that the treatments have had a real effect. In particular, the variance in the groups together with the magnitude of effect will be used in power calculations to achieve 80% power at the 95% confidence level (regarded by regulatory authorities as the standard by which such experiments should be designed).

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Studies will be performed in cattle, sheep and / or goats as appropriate. Animals of both sexes can be used for this type of animal experiment unless the study has to be performed in pregnant and / or lactating animals.

Purchase of animals: The animals will be purchased from commercial suppliers, obtained from affiliated farms or bred at [redacted] facilities.
If a certain microbial status is required, animals will be purchased from farms with the respective (certified) microbiological status and / or screened prior to inclusion in the study.
If the study design requires that the animals are vaccinated and / or infected at [redacted] or when only [redacted], the young animals have to be [redacted] accordingly from the farm of birth to the testing facility.

Special requirements
If young animals with a specific antibody status are required, it is often necessary to warrant that [redacted] As these [redacted] animals are more susceptible for intercurrent infections, they receive [redacted]. With the exception of studies against enteric pathogens, colostrum can be given to the animals from [redacted] as an aid in the prevention of [redacted]

Age of animals:
Age of the animals for vaccine research varies from a few hours old to adult. The age of the animals to

be used should be the minimal age recommended for use of the vaccine.
If very young lambs or kids have to be vaccinated, it might be necessary to include the ewes / goats to foster the lambs / kids. For vaccines intended to be used for pregnant / lactating animals, it may be necessary to include dams in one or more specific trimester of pregnancy, depending on the vaccination schedule to be recommended. Samples might have to been taken from the offspring, for certain studies, euthanasia of the offspring might be required.

The tables below, specify, which models would be used for the different pathogens that might be included in research projects over the next 5 years. The lists are more extensive than the actual portfolio will be, but it is not possible at this moment to predict, which pathogens will be worked on.
The animal categories listed are the age groups considered to be most sensitive and therefore have to be used to perform the basic efficacy studies.

Priorities within the R&D portfolio are based on market needs and the estimated likelihood of success of obtaining a vaccine candidate that fulfills the required product profile. Priorities can shift upon identification of new unmet needs in the field. For example, if a new [redacted] pathogen with substantial impact on the ruminant industry is discovered, this will be given priority over research into a second generation improved product for a [redacted] pathogen.

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 2 wk
New pathogen	Cattle <6 Months old	Severe (max 70%)[1]	Max 2 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 2 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 2 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 40%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Severe (max 50%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
[redacted]	Cattle <6 Months old	Moderate (max 70%)	Max 1 wk
New pathogen	Cattle <6 Months old	Moderate (max 100%)[2]	Max 2 wk

[1]Vaccines are given to [redacted] in order to [redacted]. This part of the study does only cause mild discomfort. The information given in the table above relates to the discomfort during challenge studies in [redacted] will be determined.

[2]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 50%)	Max 2 wk
[redacted]	Adult cows	Moderate (max 50%)	Max 2 wk
[redacted]	Adult cows	Mild	Max 2 wk
[redacted]	Adult cows	Mild	Max 2 wk
[redacted]	Adult ewes	Mild	Max 2 wk
[redacted]	Adult ewes	Mild	Max 2 wk
New pathogen	Adult cows	Moderate (max 100%)[1]	Max 2 wk
New pathogen	Adult ewes	Moderate (max 100%)[1]	Max 2 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 50%)	Max 1 wk
New pathogen	Adult cows	Severe (max 100%)[1]	Max 2 wk

[1]: Studies with potential new pathogens are given the highest expected discomfort score until the severity of clinical signs has been established

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Adult cows	Moderate (max 70%)	Max 2 wk

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	Cattle<6Months and adult cows	Moderate (max 50%)	Max 2 wk

[redacted]

Pathogen	Animal category	Discomfort of disease (% of animals with highest score)	Duration of discomfort
[redacted]	sheep <6 Months	Severe (max 40%)	Max 1-2 d
	cattle<6 Months	Moderate (max 70%)	Max 2 wk
[redacted]	sheep <6 Months	Moderate (max 70%)	Max 2 wk
[redacted]	Adult cows	Severe (max 70%)	Max 1-2 days
[redacted]	sheep <6 Months	Severe (max 50%)	Max 1 wk
[redacted]	cattle<6 Months	Moderate (max 70%)	Max 1 wk
[redacted]	sheep <6 Months	Mild	Max 2 wk
	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Mild	Max 2 wk
[redacted]	cattle<6 Months	Severe (max 50%)	Max 1-2 d
[redacted]	sheep < 6 Months	Severe (max 50%)	Max 1-2 d
[redacted]	cattle<6 Months sheep < 6 Months	Mild	Max 2 wk
New pathogens and new types of models (eg. [redacted])	cattle<6 Months sheep < 6 Months	Severe (max 100%)[2]	Max 1 w

[1] Efficacy of [redacted] vaccines in ruminants is tested by [redacted] only

[2]: Studies with potential new pathogens are given the maximum discomfort score until the severity of clinical signs has been established

Based on the experience over the last 5 years and the current R&D program and priorities, the total expected number of cattle, sheep and goat per age group and discomfort category is the following:

Species	Discomfort score*	Animals <6 Months	Adult**

Cattle	Mild	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]
Sheep	Mild	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]
Goat	Mild	[redacted]	[redacted]
	Moderate	[redacted]	[redacted]
	Severe	[redacted]	[redacted]

*: Discomfort due to disease
**: Including [redacted]
***: Due to repeated procedures the overall discomfort will be moderate in at most 50 % of the animals [redacted]

In the vaccination studies, one or more vaccinated groups are compared to an unvaccinated control group. The group size is dependent on the challenge model and the age of the animal at the time of challenge, as for some pathogens the susceptibility for infection/disease will diminish with age, making the use of relatively large group sizes necessary to be able to show a statistically significant difference. In general, the group size used will be [redacted], but could be larger depending on the expected variation in the infection model.
The expected numbers per category of vaccine are:

Per Model	Cattle <6Months	Adult cattle	Sheep <6Months	Adult sheep	Goat <6Months	Adult goat
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

For the vaccines that are currently in the research phase, challenge will still have to be performed in [redacted] of the models to prove efficacy because of the absence of an immunological correlate of protection.

C. Re-use

Will the animals be re-used?

☐ No, continue with question D.

X Yes > Explain why re-use is considered acceptable for this animal procedure.

Re-use might be considered for animals that experience only minor discomfort during the first study, for example uninfected control animals. This approach is considered acceptable especially in case of animals that were raised under special conditions (e.g. [redacted]) to achieve a certain [redacted] status.

Are the previous or proposed animal procedures classified as 'severe'?

xNo

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:
In accordance with international regulations, animals of the target species must be used to demonstrate the safety and efficacy of a vaccine because there are no suitable alternatives or models for the induction of immunity in a whole organism or for the infection of living tissues as complex as those found in the whole animal in which the vaccines are intended to have efficacy. Therefore, during the research phase, candidate vaccines have to be tested in the same models.

Reduction: All studies are performed with the lowest possible number of animals that are required to enable meaningful interpretation of the results. This will be achieved through an ongoing evaluation of the observations in each study. The number of animals per study will be substantiated in each study protocol. According to internal procedures, the study protocol will be reviewed by the Animal Welfare Body and a statistician.

Refinement:
Where possible it is pursued to refine the routes of administration of substances and sampling techniques to improve animal welfare/to reduce discomfort of administration, but without endangering the scientific outcome. Ruminants are the target species and there are no other less innervated/sentient species that could be a model for the ruminant diseases that are studied. See next paragraph for other refinement methods that are applied.
The classic method to prove protection of a new vaccine is efficacy in a vaccination-challenge test. However, if immunological correlates of protection (e.g. a serological response) can be used to prove efficacy this will be used rather than challenge infection. When an infection model has to be used, humane endpoints will be employed and staff will be fully trained to recognize animals that experience discomfort. Animals will be closely monitored and additional health checks are performed to ensure that no animal is left suffering.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

For monitoring of the clinical health status of animals, all study animals will be checked at least once a day by a certified person. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.
Furthermore efforts are made to optimally enrich the environment during containment.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

The vaccines in the companys R&D program are unique and proprietary to the company. To show that vaccines are compatible (combined or associated use), a number of the safety and efficacy studies done with the individual products has to be repeated with the vaccines administered together according to international regulations and guidelines.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[] No

X Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

The animals are housed socially, but animals might have to be housed [redacted] in order to [redacted] Some studies may require limited bedding during containment. ~~In a few cases~~ [redacted] ~~.~~ **In [redacted] infection models it is necessary to collect the [redacted] in order to perform a visual inspection and to determine the [redacted] and / or total amount of [redacted] Therefore, no substrate can be offered in this type of studies.**

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

XYes > Will anaesthesia, analgesia or other pain relieving methods be used?

[] No > Justify why pain relieving methods will not be used.

Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Vaccination, injections (for application of challenge material, injection with [redacted] drugs or agents) as well as sampling of blood are part of normal farm practice/veterinary care and will induce only mild discomfort. If the sampling is repeated (>5), the discomfort is considered to be moderate as a result of the stress when restrained and during handling of the animal.
All biotechnical procedures such as vaccination and blood sampling procedures have been described in Standard Operating Procedures (SOPs) and only well trained personnel will be responsible for the execution (GLP accredited procedures).

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Measurement of body temperatures and body weight, sampling urine, feaces, colostrum/milk, sampling on different mucosae ([redacted] as well as [redacted] are part of normal farm practice/veterinary care and will induce only mild discomfort. If the following proceudres [redacted] sampling or [redacted], punction of [redacted] punction [redacted] biopsy) are repeated the discomfort is considered to be moderate.
All biotechnical procedures such as vaccination and sampling procedures have been described in Standard Operating Procedures (SOPs) and only well trained personnel will be responsible for the execution (GLP accredited procedures).
[redacted] animals are more susceptible to disease and might therefore encounter discomfort related to intercurrent diseases. Transport of the animals to the testing facility might cause transient discomfort for the duration of the transport, especially for animals that are transported [redacted]
Vaccination can cause a transient increase in rectal temperature, sometimes accompanied with a reduced

level of activity, and a transient vaccination site reaction. Systemic reactions generally disappear within 24 hours, but local reactions (that are generally painless) can persist for several days and even weeks. The safety and efficacy profile of the vaccine compositions used in the research phase is not yet determined. Therefore, it is possible that adverse events occur or that protection of the vaccinated animals is very limited.
Depending on the nature of the challenge inoculum the discomfort of the challenge can range from mild in the absence of any clinical signs (e.g. [redacted]) to moderate (e.g [redacted] and severe (e.g. [redacted]). Vaccination will result in a significant reduction of clinical abnormalities after challenge compared to the unvaccinated control group and thus to a reduction of animals with discomfort.
In case of challenge studies with pathogens that do not cause clinical disease or only very mild disease, it might be necessary to [redacted] the animals. These procedures and any effects related to the [redacted] treatment are already taken into consideration for the discomfort levels and durations listed above in the respective tables.
The clinical health status of all animals is checked at least once a day by qualified personnel. Special attention is paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.
In consultation with the veterinarian and Study Director, if treatment does not interfere with the test results, it will be decided whether to apply adequate veterinary care including analgesia to alleviate treatment related pain (for example infection studies with Mastitis pathogens or Foot and Mouth Disease virus) or pain not related to the treatment. In case of severe suffering, humane endpoints are applicable. General humane endpoints are described in an SOP (e.g. the condition of the animal prevents it from eating and drinking regularly, severe loss of body weight) and test specific humane endpoints are given in each study protocol if applicable.

Explain why these effects may emerge.

These procedures may be part of the experimental design. For vaccines intended for use in young animals, the study design may require that the animals are vaccinated and / or infected [redacted] or when only a [redacted]. In these cases, the young animals have to be [redacted] accordingly from the farm of birth to the testing facility.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

All biotechnical procedures will only be performed according to standard procedures described in SOPs (GLP accredited procedures).
The number of samplings will be done in accordance with the respective guidelines or if no requirements are given, the number of samplings is reduced to a minimum number required to for a valid evaluation of results.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

To determine the efficacy of a vaccine it is necessary to challenge animals with the pathogenic organism. The severity of discomfort is depending on the nature of the pathogen (see 3.1 of the project proposal for specific clinical signs of the pathogens involved). However, the duration of severe discomfort will be limited due to the application of a humane endpoint if needed.
General humane endpoints are applicable to all animals, irrespectively of the type of experiment.
General Humane Endpoints:

- The animal experiences more than minor additional discomfort as a consequence of conditions resulting in long term or non-reversible inability to eat and or drink autonomously, fast or long lasting loss of weight, diseases or conditions that cause severe pain, suffering or discomfort such as bone fractions, force unnatural positioning and / or movements, open wounds or absesses.
- Scientific endpoints: The target of the study reached / all planned samplings have been performed
- (Reliable and useful) results cannot be reached for reasons unrelated to the study

Specific humane endpoints after infection with bovine pathogens

- In general, disease specific clinical signs (for example [redacted] or [redacted] clinical signs) do not normally lead to humane endpoints, but they may affect the general health of the animal (i.e. high fever, dullness, anorexia) leading to humane endpoints if
  - severe general clinical signs last for at least 2 days or the body temperature drops rapidly
  - the animal is unable to stand up and eat/dring actively for more than 1 day
- In the [redacted] are humane endpoints.

These test-specific humane endpoints need to be described in the corresponding study protocol. Each study protocol is reviewed by the AWB before execution of the study.
In case it is difficult to reach a decision based on the pre-defined criteria for an endpoint the designated veterinarian is empowered to decide that a humane endpoint is applied/reached.

Indicate the likely incidence.

Considering the expected number of studies with the different pathogens, the expected number of animals included in the different treatment groups (i.e. vaccinated / infected / control group) and the expected severity, at most 20 % of the animals is expected to have severe discomfort that would require euthanasia.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

For studies without challenge, discomfort will be mild to moderate depending on the number of sampling points. For vaccination-challenge studies, the type and severity of the clinical signs are depending on the type of challenge infection. Similar to natural field infections they may cause mild to severe pain, distress, suffering or even impending death. See B for an overview of the different pathogens involved, the maximal discomfort caused by the disease and the maximum number of animals expected to reach the highest discomfort category. Vaccination is expected to reduce the level of discomfort after challenge, but the non-vaccinated control group will experience the symptoms of the natural infection.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Postmortem investigation can be part of the experimental design to evaluate (histo)pathological lesions at the injection sites and or to evaluate effect of the infection on different organ systems and or to attempt re-isolation of the inoculum from tissues and organs.
In addition, animals vaccinated with a non-licensed vaccine or infected with a pathogen cannot be returned to the farm of origin or transported to another farm to prevent the spread of disease. Therefore, all animals might have to be euthanized at the end of the study or when a humane endpoint is reached. Control animals that have not been vaccinated and infected may be reused or returned to the farm of origin or transported to another farm. In case of a surrogate immunological marker (no challenge), animals housed on contract farms can remain on the farm or transported to other farms until the end of their natural/economic life.
Moreover, return of the animals to commercial farms or slaughter for human consumption is often prohibited by the current legislation on use of antibiotics.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	22100
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 3 Type of animal procedure: Research: Assay development and preparation of biomaterials

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

The potency of each inactivated vaccine batch used in development studies has to be determined to set the limits for release and to determine the stability. Traditionally, the potency of inactivated vaccines, is measured in a so-called *in vivo* potency in [redacted]. During the research phase of a vaccine project, the test protocol for the potency test is determined. In an initial study, the strenghth of the immune reponse is compared between the different animal species. In further studies, it is investigated, whether the antibody response is dose dependent and the release criteria are determined.
Apart from the replacement of experimental animals, *in vitro* potency tests are very much preferred over *in vivo* testing for multiple other reasons including costs and timelines. However, for several inactivated vaccines, *in vivo* potency tests are still necessary because it is either not possible to determine the antigen and/or adjuvant content *in vitro* or an *in vivo* batch test is mandatory under the respective legislation (e.g. Ph.Eur monograph.).
In such a batch potency test, animals are injected with one or more doses of the vaccine batch. The potency is preferably determined by measuring the antibody response. In a few cases, determination the protection against challenge infection is mandatory or necessary for scientific / technical reasons.
Biomaterials such as specific antisera or monoclonal antibodies are needed in most vaccine projects for different purposes such as the identification, characterisation and quantification of the vaccine strain, neutralizing the vaccine virus, setting up *in vitro* tests et cetera.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Two or more of the following treatments will be employed in order to fulfil the requirements for the potency studies as laid down in the Ph Eur *(in italics the frequency of the treatments).* The same

treatments are also applied for the preparation of biomaterials*:*
1. Blood sampling [redacted] to determine [redacted] parameters
2. Test substance administration [redacted]
3. Weighing [redacted]
4. Euthanasia
The duration of these procedures will only be minutes.
In case of potency tests that involve challenge infection, the following additional treatments are employed
5. Administration of challenge material [redacted]
6. Clinical observation [redacted]

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

The group sizes for potency tests that are specified in guidelines typically range between 5 and 10. For those models that are not described in specific regulations, a group size of 5-10 animals is generally accepted by regulatory authorities. If sufficient knowledge on the variation is available the group size will be calculated such that a statistically significant difference between standard and substandard vaccine batches can be made with 80% power and 95% confidence.
For the preparation of antisera the minimal number of animals that will provide the required volume will be used.
For the preparation of monoclonal antibodies, 5 mice will be used per antigen.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

For some vaccines, the species to be used for a batch potency test is prescribed in a Ph.Eur monograph.
In general, [redacted] are preferred over the ruminant target species because they are more genetically homogeneous and better microbiologically controlled.
Therefore, less variance in response and better reproducibility can be achieved, which means that the number of experimental animals can be lower than when using ruminants.
For some vaccines, the type of laboratory animals to be used for a batch potency test is prescribed in a Ph.Eur monograph.
Preparation of antisera in non-ruminant animals or murine monoclonal antibodies has the advantage, that these animals are free of antibodies against other ruminant pathogens.
Based on the experience over the last 5 years and the current R&D program and priorities, the total expected number of laboratory animals is the following:
[redacted]
of both sexes will be used, but for [redacted] only females will be included in studies because of a higher risk of fighting in male animals and the relatively long time that the animals are in an experiment. With regard to the length of these experiments (1-2 months) it is not acceptable to use males, because they would need to be single housed.
Welfare concerns are the basis for the preferred use of female [redacted] because they hardly fight. Data from recently executed experiments with male animals have shown that in half of the experiments there was a loss of animals because of severe fighting. This has resulted in the repetition of these experiments and therefore in the use of more animals. A loss of animals due to fighting has never occurred when female animals were used. We consider the aggression and fighting a worrying impairment of welfare. Moreover, the aggression will cause stress, which is known to have effects on the immune system.
In addition, use of animals of both sexes would increase the variablity and thereby increase the number of animals needed.
In these studies the functioning of the immune system is crucial and variation in the immune response

caused by external factors should be avoided as much as possible.

Origin [redacted]:
All animals are supplied by certified vendors accompanied by a health certificate according to FELASA recommendations. All purchased animals have a SPF status.

Origin [redacted]
Own [redacted] breeding unit or commercial vendor.

Age of animals:
The required species and age (or weight) is usually designated as the most sensitive species or age for the test component in question. If such specific knowledge is not available the most practical choices are made, based on possibilities for purchase and housing conditions. Moreover, animals must be immunologically fit to be subjected to immunizations and blood samplings.

C. Re-use

Will the animals be re-used?

X No, continue with question D.

Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

XNo

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:
Apart from the replacement of experimental animals, *in vitro* potency tests are very much preferred over *in vivo* testing for multiple other reasons including costs and timelines. Therefore, multidisciplinary teams are active at the company to replace *in vivo* potency tests by *in vitro* tests such as antigenic mass assays for the potency testing of vaccines.

Reduction: The animal species that is expected to give the most discriminatory test with the smallest number of animals will be used. The number of animals per study will be substantiated in each study protocol. Each study protocol will be reviewed by the Animal Welfare Body and a statistician.

Refinement: Following the codes of practice for immunization is the basis for refinement in these animal procedures. Where possible it is pursued to refine the routes of administration of substances and sampling techniques to improve, but without endangering the scientific outcome. For example; blood sampling in rodents is done under anesthesia. See next paragraph for other refinement methods that are applied.
Where ever possible, potency testing will be based on the testing of antibodies or other correlates of protection rather than by challenge.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Following the code of practice for immunization and the code of practice for monitoring the welfare of the animals is the basis for refinement in these animal procedures.
In general animals are always housed socially, but animals might have to be (temporarily) separated due to fighting or because of veterinary concerns. Furthermore, to enhance animal welfare, species specific environmental enrichment is provided to all animals.

For monitoring of the clinical health status of animals, all study animals will be checked at least once a day by a certified person. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

A potency test model needs to be developed for a specific vaccine. Experiences from vaccines with similar composition can be used, but eventually, the model has to be validated for the specific vaccine. Specific biomaterials have to be available for each project. Prior to the preparation of new biomaterials, the scintific literature and the company database for biomaterials available at the different research sites will be consulted in order to prevent unnecessary preparation of new biomaterials.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[] No

X Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

Some studies may require limited bedding during containment.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

XYes > Will anaesthesia, analgesia or other pain relieving methods be used?

X No > Justify why pain relieving methods will not be used.

X Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Injections (vaccination, inoculation of challenge material) and blood sampling are part of normal veterinary care / commonly used biotechnical procedures and will induce only mild discomfort or moderate discomfort of very short duration.

Anesthesia will be applied during blood sampling of mice, guinea pigs and rats. Anesthesia will not be applied during sampling blood from chickens and rabbits. In these species blood sampling causes only

mild discomfort or moderate discomfort of very short duration. Applying anesthesia would only increase discomfort as the animal needs to be restrained during administration.

For each species blood sampling and other biotechnical procedures have been described in Standard Operating Procedures (SOPs) (GLP accredited procedures).
As the vaccines to be tested have already been found to be safe in the target species (ruminants) it is very unlikely that they will cause adverse effects in non-target animals.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

In general, biotechnological procedures such as weighing and sampling may result in discomfort, because animals need to be fixated and especially if performed repeatedly.
Moreover, application of the vaccine can result in a transient increase in rectal temperature, sometimes accompanied with a reduced level of activity, and a transient vaccination site reaction. Systemic reactions will generally disappear within 24 hours, but local reactions (swelling redness), that are generally painless, can persist for several days and even weeks, but these local reactions do not affect normal behaviour (activity, feeding and drinking).
Depending on the nature of the challenge inoculum, the discomfort of the challenge can range from mild in the absence of any clinical signs (e.g. [redacted]) to moderate (e.g [redacted] and severe (e.g. [redacted]). Vaccination will result in a significant reduction of clinical abnormalities after challenge compared to the unvaccinated control group.
For monitoring of the clinical health status of animals, all study animals will be checked at least once a day by a certified person. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed.
In case animals experience discomfort (whether or not related to the treatment), it will be decided in consultation with the veterinarian and Study Director whether to apply adequate veterinary care to alleviate unexpected pain and/or distress (if treatment does not interfere with the test results). In case of severe suffering, humane endpoints are applicable. General humane endpoints are described in an SOP (e.g. the condition of the animal prevents it from eating and drinking regularly, severe loss of body weight) and test specific humane endpoints are given in each study protocol if applicable.

Explain why these effects may emerge.

These procedures and if applicable the clinical signs caused by the challenge may be part of the experimental design.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Weighing is part of normal veterinary care and will induce only mild discomfort. Taking samples of mucosal surfaces or urine will result in mild discomfort, with the exception of repeated mucosal swabbing, which is considered to be moderate discomfort. Biotechnical procedures have been described in SOPs (GLP accredited procedures). Unless required by the applicable regulations or scientific reasons, potency testing will be based on the testing of correlates of protection such as antibody levels rather than by challenge.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

In potency tests that require challenge with a pathogenic organism, the severity of discomfort is depending on the nature of the pathogen. However, the duration of severe discomfort will be limited due to the application of a humane endpoint if needed.
General humane endpoints are applicable to all animals, irrespectively of the type of experiment.
General Humane Endpoints:

- The animal experiences more than minor additional discomfort as a consequence of conditions resulting in long term or non-reversible inability to eat and or drink autonomously, fast or long lasting loss of weight, diseases or conditions that cause severe pain, suffering or discomfort such

as bone fractions, force unnatural positioning and / or movements, open wounds or absesses.
- Scientific endpoints: The target of the study reached / all planned samplings have been performed
- (Reliable and useful) results cannot be reached for reasons unrelated to the study

Specific humane endpoints after infection with bovine pathogens

- In general, disease specific clinical signs (for example [redacted] clinical signs) do not normally lead to humane endpoints, but they may affect the general health of the animal (i.e. high fever, dullness, anorexia) leading to humane endpoints if
  - severe general clinical signs last for at least 2 days or the body temperature drops rapidly
  - the animal is unable to stand up and eat/dring actively for more than 1 day

These test-specific humane endpoints are described in the corresponding study protocol. Each study protocol is reviewed by the AWB before execution of the study.
In case it is difficult to reach a decision based on the pre-defined criteria for an endpoint the designated veterinarian is empowered to decide that a humane endpoint is applied/reached.

Indicate the likely incidence.

As the overall majority of potency tests as well as the preparation of biomaterials do not require challenge, the incidence of severe discomfort requiring euthanasia is estimated to be less than 2 %.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Discomfort will be mild (≥75%) to moderate (≤25%) depending on the number of sampling points, type of biotechnical procedure and whether the challenge infection causes disease in the model animal.
Challenge infection will be performed in [redacted] of the animals.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

For some potency models it is necessary to investigate the presence of (histo)pathological lesions and take organ and tissue samples for re-isolation of the vaccine strain.
For the preparation of antisera it is necessary to kill the animals in order to obtain the required volumes.
For preparation of monoclonal antibodies mice are killed to collect the lymphoid organs that are needed

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

19.

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

[redacted]

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD2210020171629
Bijlagen
1

Datum 14 juni 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

[redacted]

Op 2 mei 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Research of new ruminant vaccines" met aanvraagnummer AVD2210020171629. Wij hebben uw aanvraag beoordeeld.

Op 23 mei en 9 juni 2017 heeft u uw aanvraag aangevuld. Dit betrof meer achtergrondinformatie over de infecties, onderbouwing van de keuzemomenten, de reden van afwijkende huisvesting, het te verwachten ongerief van de dieren, onderbouwing van het gebruik van vrouwelijke dieren, betere beschrijving van de humane eindpunten, het totaal aantal te onderzoeken vaccins, het aantal nakomelingen dat gebruikt wordt in het project, het ontbreken van bedding in sommige studies en het aantal dieren dat een challenge zal ondergaan.

Beslissing
Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de Dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning.
De algemene voorwaarde(n) zijn opgenomen op grond van artikel 1d lid 4, artikel 10a1 lid 2, artikel 10 lid 2 en/of artikel 10a3 van de wet.
Er is gevraagd om wetenschappelijk te onderbouwen waarom niet beide geslachten gebruikt kunnen worden. Uw antwoord hierop is niet voldoende onderbouwd, daarom moeten voor Bijlage Dierproeven 3.4.4.3 beide geslachten in evenredige aantallen gebruikt worden.

U kunt met uw project "Research of new ruminant vaccines" starten. De vergunning wordt afgegeven van 1 september 2017 tot en met 31 augustus

2022. Deze termijn is anders dan in uw aanvraag, omdat een vergunning een looptijd van maximaal 5 jaar kan hebben.

[redacted]
vaccins te [redacted]

Voor het vervoer van jonge of drachtige dieren wordt Bijlage I, Hoofdstuk 1 van de Europese Transportverordening (EG nr. 1/2005) in acht genomen. Overige wettelijke bepalingen blijven van kracht.

Beoordeling achteraf
Na afloop van het project zal er een beoordeling plaatsvinden, zoals bedoeld in artikel 10a1, lid 1d en lid 3, in de wet. Meer informatie over de eisen bij een beoordeling achteraf vindt u in de bijlage.
Er is sprake van ernsig ongerief.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie [redacted] gevoegd. Dit advies is opgesteld op 2 mei 2017. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet.
In aanvulling op het DEC-advies stelt de CCD voorwaarden. De voorwaarden staan in de vergunning beschreven. Voor het overige nemen wij het advies van de DEC over, inclusief de daaraan ten grondslag liggende motivering.
Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

Bezwaar
Als u het niet eens bent met deze beslissing, kunt u binnen zes weken na verzending van deze brief schriftelijk een bezwaarschrift indienen.
Een bezwaarschrift kunt u sturen naar Centrale Commissie Dierproeven, afdeling Juridische Zaken, postbus 20401, 2500 EK Den Haag.

Bij het indienen van een bezwaarschrift vragen we u in ieder geval de datum van de beslissing waartegen u bezwaar maakt en het aanvraagnummer te vermelden. U vindt deze nummers in de rechter kantlijn in deze brief.

Bezwaar schorst niet de werking van het besluit waar u het niet mee eens bent. Dat betekent dat dat besluit wel in werking treedt en geldig is. U kunt tijdens deze procedure een voorlopige voorziening vragen bij de Voorzieningenrechter van de rechtbank in de woonplaats van de aanvrager. U moet dan wel kunnen aantonen dat er sprake is van een spoedeisend belang.

Voor de behandeling van een voorlopige voorziening is griffierecht verschuldigd. Op http://www.rechtspraak.nl/Organisatie/Rechtbanken/Pages/default.aspx kunt u zien onder welke rechtbank de vestigingsplaats van de aanvrager valt.

Datum:
14 juni 2017
Aanvraagnummer:
AVD2210020171629

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Centrale Commissie Dierproeven
namens deze:

[redacted]

Algemeen Secretaris

Bijlagen:
- Vergunning
  Hiervan deel uitmakend:
  - DEC-advies
  - Weergave wet- en regelgeving

Centrale Commissie Dierproeven

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan
Naam: [redacted]
Adres: [redacted]
Postcode en plaats: [redacted]
Deelnemersnummer: [redacted]

deze projectvergunning voor het tijdvak 1 september 2017 tot en met 31 augustus 2022, voor het project "Research of new ruminant vaccines" met aanvraagnummer AVD2210020171629, volgens advies van Dierexperimentencommissie [redacted]. Hierbij is afgeweken van het DEC-advies. Er worden aanvullende voorwaarde(n) gesteld. Beide geslachten moeten worden gebruikt in Bijlage 3.4.4.3. Daarnaast is het enkel toegestaan tegen de in het projectvoorstel genoemde pathogenen vaccins te ontwikkelen. [redacted]
De functie van de verantwoordelijk onderzoeker is [redacted]
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 2 mei 2017
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 23 mei 2017;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 23 mei 2017;
c Advies van dierexperimentencommissie d.d. 2 mei 2017, ontvangen op 2 mei 2017.
d De aanvullingen op uw aanvraag, ontvangen op 23 mei en 9 juni 2017

Aanvraagnummer:
AVD2210020171629

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
3.4.4.1 Research: Infection studies in ruminants				Runderen: [redacted] kalveren < 6 maanden; [redacted] volwassen dieren Schapen: [redacted] lammeren < 6 maanden; [redacted] volwassen dieren Geiten: [redacted] lammeren < 6 maanden; [redacted] volwassen dieren
	Runderen (Bos taurus) /	[redacted]	[redacted] Ernstig [redacted] Matig [redacted] Licht	
	Schapen (Ovis aries) /	[redacted]	[redacted] Ernstig [redacted] Matig [redacted] Licht	
	Geiten (Capra aegagrus hircus) /	[redacted]	[redacted] Ernstig [redacted] Matig [redacted] Licht	
3.4.4.2 Research: Vaccination challenge studies in ruminants				

Aanvraagnummer:
AVD2210020171629

	Runderen (Bos taurus) /	[redacted]	[redacted] Ernstig [redacted] Matig [redacted] Licht	
	Schapen (Ovis aries) /	[redacted]	[redacted] Ernstig [redacted] Matig [redacted] Licht	
	Geiten (Capra aegagrus hircus) /	[redacted]	[redacted] Ernstig [redacted] Matig [redacted] Licht	
3.4.4.3 Research: Assay development and preparation of biomaterials				
	Konijnen (Oryctolagus cuniculus) /	700	[redacted] Matig [redacted] Licht	
	Muizen (Mus musculus) /	650	[redacted] Matig [redacted] Licht	
	Ratten (Rattus norvegicus) /	650	[redacted] Matig [redacted] Licht	

Aanvraagnummer:
AVD2210020171629

	Cavia's (Cavia porcellus) /	550	[redacted] Matig [redacted] Licht	
	Kippen /	700	[redacted] Matig [redacted] Licht	

Voorwaarden

Op grond van artikel 10a1 lid 2 van de Wet op de dierproeven zijn aan een projectvergunning voorwaarden te stellen
In dit project worden dierproeven toegepast die vallen in de categorie ernstig volgens artikel 10b van de wet en wordt daarom voorzien van beoordeling achteraf.Deze beoordeling zal uiterlijk december 2022 plaatsvinden. Er zal dan beoordeeld worden of de doelstellingen van het project werden bereikt. Daarnaast wordt bekeken of de schade die de dieren hebben ondervonden, het aantal en soorten proefdieren en de ernst de dierproeven conform de vergunning waren.

Gedurende de looptijd van de vergunning, koppelt de aanvrager aan de CCD terug welk soort vaccin tegen welke aandoening in welk type dierproef met wijze van uitvoering in welke diersoort en bijbehorend ongerief is uitgevoerd onder deze vergunning. Deze terugkoppeling moet uiterlijk 31 januari door de CCD ontvangen zijn en rapporteert over het afgelopen kalenderjaar (1 januari - 31 december). Ook wanneer er geen dierstudies zijn uitgevoerd wordt dit gerapporteerd. De CCD kan op basis van deze terugkoppeling aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarden wijzigen of intrekken.
Wanneer u overtuigend en onbetwistbaar kan aantonen dat er geen gegevens over de geteste stof kunnen worden vrijgegeven omdat de opdrachtgever deze als vertrouwelijke informatie heeft geclassificeerd kunt u deze informatie buiten de rapportage houden.

In artikel 10, lid 1 sub a van de wet, wordt bepaald dat het verboden is een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook kan worden bereikt anders dan door middel van een dierproef, of door middel van een dierproef waarbij minder dieren kunnen worden gebruikt of minder ongerief wordt berokkend dan bij de in het geding zijnde proef het geval is. Nieuwe onderzoeken naar alternatieven kunnen tot gevolg hebben dat inzichten en/of omstandigheden van het aangevraagde project in de vergunningsperiode wijzigen, gedurende de looptijd van deze vergunning.

Indien bovenstaande zich voordoet dient aanvrager dit in afstemming met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarde wijzigen of intrekken.

Mannelijke en vrouwelijke dieren moeten in evenredige aantallen gebruikt worden in Bijlagen Dierproeven 3.4.4.3, tenzij het onderzoek naar [redacted]. Indien gedurende het project blijkt dat er geslachts-specifieke effecten zijn, kunt u deze informatie als wijziging rapporteren aan de CCD. Deze informatie kan voor de CCD aanleiding zijn om bovenstaande voorwaarde van gelijk gebruik van beide geslachten te wijzigen of in te trekken.

Centrale Commissie Dierproeven

Aanvraagnummer:
AVD2210020171629

Weergave wet- en regelgeving

Dit project en wijzigingen
Volgens artikel 10c van de Wet op de Dierproeven (hierna de wet) is het verboden om andere dierproeven uit te voeren dan waar de vergunning voor is verleend. De dierproeven mogen slechts worden verricht in het kader van een project, volgens artikel 10g. Uit artikel 10b volgt dat de dierproeven zijn ingedeeld in de categorieën terminaal, licht, matig of ernstig. Als er wijzigingen in een dierproef plaatsvinden, moeten deze gemeld worden aan de Centrale Commissie Dierproeven. Hebben de wijzigingen negatieve gevolgen voor het dierenwelzijn, dan moet volgens artikel 10a5 de wijziging eerst voorgelegd worden en mag deze pas doorgevoerd worden na goedkeuren door de Centrale Commissie Dierproeven.
Artikel 10b schrijft voor dat het verboden is een dierproef te verrichten die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, tenzij hiervoor door de Minister een ontheffing is verleend.

Verzorging
De fokker, leverancier en gebruiker moeten volgens artikel 13f van de wet over voldoende personeel beschikken en ervoor zorgen dat de dieren behoorlijk worden verzorgd, behandeld en gehuisvest. Er moeten ook personen zijn die toezicht houden op het welzijn en de verzorging van de dieren in de inrichting, personeel dat met de dieren omgaat moet toegang hebben tot informatie over de in de inrichting gehuisveste soorten en personeel moet voldoende geschoold en bekwaam zijn. Ook moeten er personen zijn die een eind kunnen maken aan onnodige pijn, lijden, angst of blijvende schade die tijdens een dierproef bij een dier wordt veroorzaakt. Daarnaast zijn er personen die zorgen dat een project volgens deze vergunning wordt uitgevoerd en als dat niet mogelijk is zorgen dat er passende maatregelen worden getroffen.
In artikel 9 staat dat de persoon die het project en de dierproef opzet deskundig en bekwaam moet zijn. In artikel 8 van het Dierproevenbesluit 2014 staat dat personen die dierproeven verrichten, de dieren verzorgen of de dieren doden, hiervoor een opleiding moeten hebben afgerond.
Voordat een dierproef die onderdeel uitmaakt van dit project start, moet volgens artikel 10a3 van de wet de uitvoering afgestemd worden met de instantie voor dierenwelzijn.

Pijnbestrijding en verdoving
In artikel 13 van de wet staat dat een dierproef onder algehele of plaatselijke verdoving wordt uitgevoerd tenzij dat niet mogelijk is, dan wel bij het verrichten van een dierproef worden pijnstillers toegediend of andere goede methoden gebruikt die de pijn, het lijden, de angst of de blijvende schade bij het dier tot een minimum beperken. Een dierproef die bij het dier gepaard gaat met zwaar letsel dat hevige pijn kan veroorzaken, wordt niet zonder verdoving uitgevoerd. Hierbij wordt afgewogen of het toedienen van verdoving voor het dier traumatischer is dan de dierproef zelf en het toedienen van verdoving onverenigbaar is met het doel van de dierproef. Bij een dier wordt geen stof toegediend waardoor het dier niet meer of slechts in verminderde mate in staat is pijn te tonen, wanneer het dier niet tegelijkertijd voldoende verdoving of pijnstilling krijgt toegediend, tenzij wetenschappelijk gemotiveerd. Dieren die pijn

kunnen lijden als de verdoving eenmaal is uitgewerkt, moeten preventief en postoperatief behandeld worden met pijnstillers of andere geschikte pijnbestrijdingsmethoden, mits die verenigbaar zijn met het doel van de dierproef. Zodra het doel van de dierproef is bereikt, moeten passende maatregelen worden genomen om het lijden van het dier tot een minimum te beperken.

Einde van een dierproef
Artikel 13a van de wet bepaalt dat een dierproef is afgelopen wanneer voor die dierproef geen verdere waarnemingen hoeven te worden verricht of, voor wat betreft nieuwe genetisch gemodificeerde dierenlijnen, wanneer bij de nakomelingen niet evenveel of meer, pijn, lijden, angst, of blijvende schade wordt waargenomen of verwacht dan bij het inbrengen van een naald. Er wordt dan door een dierenarts of een andere ter zake deskundige beslist of het dier in leven zal worden gehouden. Een dier wordt gedood als aannemelijk is dat het een matige of ernstige vorm van pijn, lijden, angst of blijven schade zal blijven ondervinden. Als een dier in leven wordt gehouden, krijgt het de verzorging en huisvesting die past bij zijn gezondheidstoestand.
Volgens artikel 13b moet de dood als eindpunt van een dierproef zoveel mogelijk worden vermeden en vervangen door in een vroege fase vaststelbare, humane eindpunten. Als de dood als eindpunt onvermijdelijk is, moeten er zo weinig mogelijk dieren sterven en het lijden zo veel mogelijk beperkt blijven.
Uit artikel 13d volgt dat het doden van dieren door een deskundig persoon moet worden gedaan, wat zo min mogelijk pijn, lijden en angst met zich meebrengt. De methode om te doden is vastgesteld in de Europese richtlijn artikel 6.
In artikel 13c is vastgesteld dat proefdieren geadopteerd kunnen worden, teruggeplaatst in hun habitat of in een geschikt dierhouderijsysteem, als de gezondheidstoestand van het dier het toelaat, er geen gevaar is voor volksgezondheid, diergezondheid of milieu en er passende maatregelen zijn genomen om het welzijn van het dier te waarborgen.
De Minister heeft vrijstelling ontheffing verleend volgens artikel 13c, die de afwijkende methode van doden op basis van wetenschappelijke motivering ten minste even humaan acht als de in de richtlijn opgenomen passende methoden.

Beoordeling achteraf
Volgens artikel 10a1, lid 1d en lid 3 van de wet worden projecten waarbij niet-menselijke primaten worden gebruikt, projecten die als ernstig ingedeelde dierproeven omvatten of een dierproef die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, achteraf beoordeeld worden.